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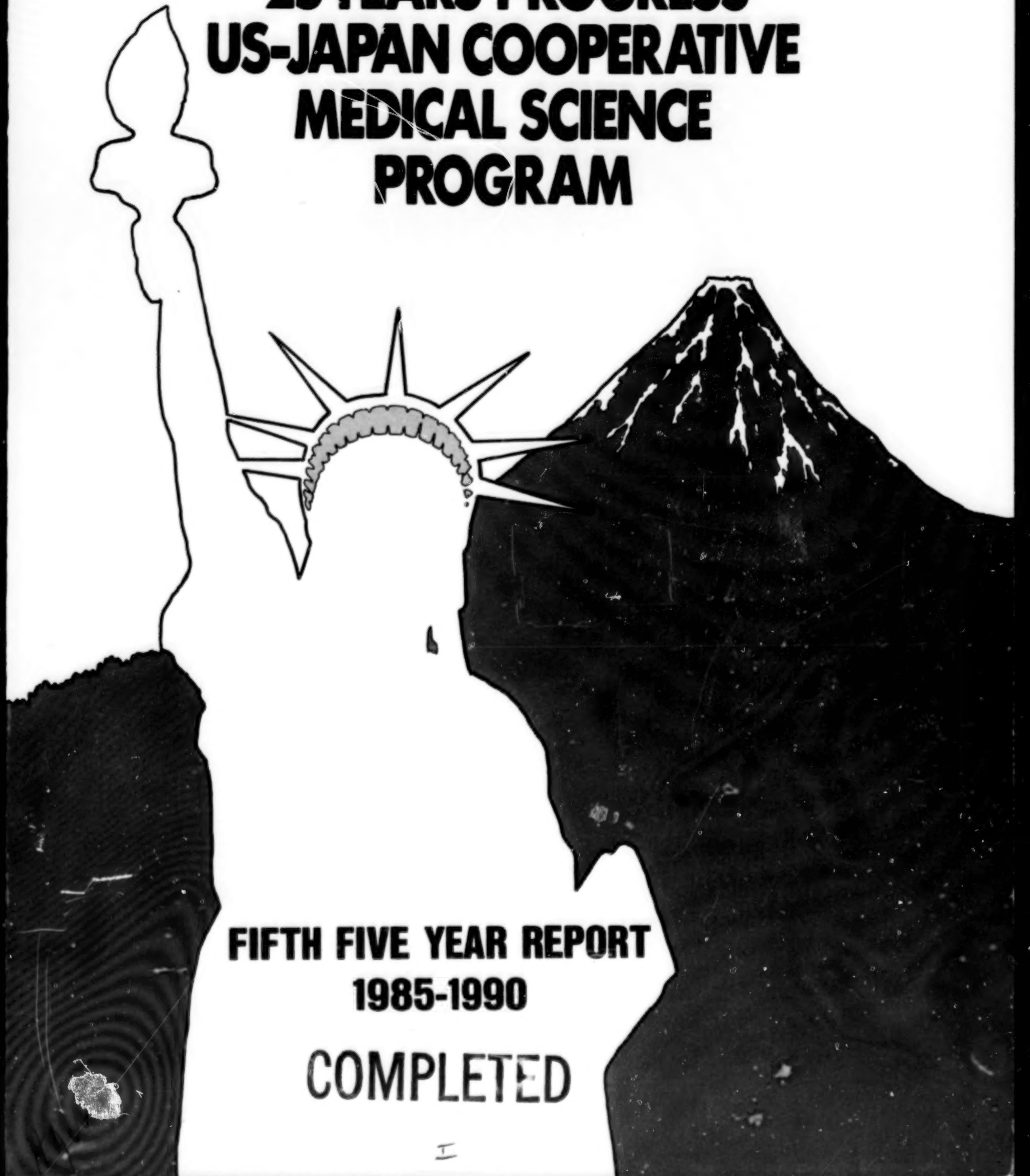
25 YEARS PROGRESS US-JAPAN COOPERATIVE MEDICAL SCIENCE PROGRAM



FIFTH FIVE YEAR REPORT
1985-1990

COMPLETED

I

The United States-Japan Cooperative Medical Science Program

Fifth Five-Year Report
1986–1990

DEPARTMENT OF STATE PUBLICATION 9761

Bureau of Oceans and International Environmental
and Scientific Affairs

Released July 1990

THE WHITE HOUSE
WASHINGTON

April 23, 1990

I am delighted to extend warm greetings and congratulations to all those involved with the United States - Japan Cooperative Medical Science Program on the occasion of its 25th anniversary.

This program stands today as a shining example of the remarkable accomplishments that are possible when two nations join together in a common endeavor.

Established in 1965 by President Johnson and Prime Minister Sato, this research program has done an outstanding job of uniting biomedical scientists from both countries in a concerted effort to eliminate widespread disease in Asia.

The research of many talented scientists has resulted in significant contributions to this joint endeavor. For example, scientific knowledge of practical importance has been produced to fight such devastating problems as AIDS, cholera, hepatitis, parasitic diseases, viral diseases, and malnutrition.

I look forward to the continued success of this flourishing science program. Barbara joins me in sending best wishes for every future success. God bless you.

George Bush

OFFICE OF THE PRIME MINISTER
TOKYO

MESSAGE FOR
THE U.S.-JAPAN COOPERATIVE MEDICAL SCIENCE PROGRAM

On behalf of the Government and people of Japan, I would like to extend our heartfelt congratulations on the 25th anniversary of the U.S.-Japan Cooperative Medical Science Program, which has been growing while expanding its study fields to meet with the requirements of the times since its establishment in 1965 in accordance with the Joint Communique between President Johnson and Prime Minister Sato.

This program, participated by a number of leading medical scientists from both countries, has contributed to the improved human health not only in Asia but also in the entire world by making a constant progress towards its worthy cause of eliminating diseases prevailing in Asia.

The publication of the program's Fifth Five-Year Report will provide a good opportunity for sharing the program's major achievements over the past twenty-five years with a large number of people. I believe the report will receive a wide public admiration.

Such admiration to these achievements is to be accredited to those who have devoted themselves to this program, to which I wish to express my deepest gratitude and respect. Such achievements also represent examples of the fruit of close cooperation between our two nations for the greater prosperity and well-being of the entire world.

I am, therefore, very proud to note that this splendid program between our two nations has been carried out so successfully for as long as a quarter of a century. I am confident that it will continuously play a valuable role in advancing welfare of all mankind.

Toshiki Kaifu

Toshiki Kaifu
Prime Minister of Japan

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PREFACE

There are several very cogent reasons for the U.S.-Japan Cooperative Medical Science Program having successfully reached its 25th anniversary. Seldom are government-sponsored programs able to achieve such a sustained period of productivity. This is true particularly of those that are international or binational in their organizational structure.

The U.S.-Japan Program was based on a firm foundation that was practically as solid as rock. It was conceived on the noble premise of helping improve the health and living standards of the impoverished and those in need in Asian countries. It was the Lord Buddha who professed that "freedom from disease is the first blessing." Perhaps the second blessing might be freedom from hunger and, incidentally, the U.S.-Japan Program has addressed itself to the problem of nutrition.

Any venture, regardless of its nature, is no better than the people who are responsible for its conduct. Leadership and wise and careful planning are really the trademarks of this remarkable effort in preventive and curative medicine that now extends over many years. Each of the two countries, Japan and the United States, selected the very best persons available to assume these heavy responsibilities. No better choices could have been made than Doctors MacLeod and Kurokawa and those who assisted them, to plan and devise the basic organizational and developmental plan. They and those to follow used objectivity, wisdom, and practical sense in selecting and recruiting the right, capable, and productive medical scientists to tackle problems of the utmost importance and complexity.

Furthermore, in Japan and the United States, each national program was given the important presence and support of established agencies. In Japan, the Ministries of Health and Education and the National Institutes of Health were immediately involved and given responsibility for oversight. In the United States, the umbrella of the Department of State and the generous resources of the National Institutes of Health ensured success.

Two simple criteria were on the side of success. One was NEED in the form of improved help, particularly for the impoverished. The second was a wholesome and intensive DESIRE on the part of these two great nations to help achieve this high aim. NEED and DESIRE were the essential ingredients.

Within the organizational structure of the program a system of peer review was devised to ensure that research remains relevant to the overall mission of improved health care. Nothing has been static. Directives for specific panel research programs have been redeveloped, modified, or completed. In some instances, entirely new specific objectives have been initiated through the organization of new panels. Changes have been made, not for the sake of change, but for more relevant approaches to problems as new technologies have become available.

This report represents 5 additional years of important medical progress under the U.S.Japan Cooperative Medical Science Program, from 1985 to 1990. Also, it highlights the unique "FIRST" scientific achievements in any specific field to the medical literature and, hence, to medical practice. It is true that the research contributions of today become the standards of medical practice in just a few years. Additionally, in this Silver Anniversary publication, many of those persons who made a singular contribution to the sum of activities are given brief but justified recognition.

During its coming years, the future is bright in terms of productive relevant research. Much needs to be done. With solution of some problems new ones appear; the cycle is seldom complete. Better vaccines, newer concepts of biologic means of protection, and more advanced therapeutic regimens are always welcome. Improvement in these areas is essential if progress is to be made. New diseases or aberrant forms of older scourges occasionally pose threats when least expected.

The United States and Japan, with their combined efforts and positive action in this unique Program, have shown how the underprivileged public can benefit by enlightened binational interaction.

Ivan L. Bennett, Jr.

Shiro Someya

In addition to the joint United States-Japan editorial effort that went into the drafting of this document, acknowledgment is made of our extensive collaboration in the preparation of this scientific report to the

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
NATIONAL INSTITUTES OF HEALTH
PUBLIC HEALTH SERVICE
DEPARTMENT OF HEALTH AND HUMAN SERVICES

DEDICATION

Norio Suwa, M.D.

Dr. Norio Suwa became a member of the Japanese Delegation of the U.S.-Japan Cooperative Medical Science Program in 1974, and served as its Chairman from 1979 to 1987, succeeding Dr. Toshio Kurokawa, the first Chairman of the Japanese Delegation. Much scientific progress and major changes occurred in the Program during Dr. Suwa's chairmanship. As in most organizations, whenever new initiatives are proposed or changes in organizational structure are attempted, many difficulties must be faced and overcome. By virtue of Dr. Suwa's broad perspectives on medical science and his practical approach, he was highly successful in solving difficult problems. Under his excellent leadership, the Hepatitis Panels and Immunology Boards were added to the Program.

On occasion of the 25th Anniversary of the U.S.-Japan Cooperative Medical Science Program, we proudly dedicate this Fifth Five-Year Report to Dr. Norio Suwa.

It is our sincere wish that Dr. Suwa will enjoy good health, which he has regained following major surgery, and will continue to contribute his sound advice so necessary for the future success of the Program.

Profiles

U.S. DELEGATION CHAIRMEN

Dr. Colin M. MacLeod, Chairman 1965–1972

Without the dedicated work and wise vision of Colin MacLeod, it is very doubtful that the U.S.-Japan Cooperative Medical Science Program would have succeeded. It was his responsibility from the U.S. side to organize and implement a suitable and practical workable medical science program, which was conceived to bring together the best investigative talents from our respective countries. The political climate in 1965, when the program began, was unsteady and troubled.

But Dr. MacLeod was not an ordinary person, and he was ideally suited for the task. Thoroughly schooled in medicine, biology, and immunology at McGill University, the Rockefeller Institute and New York University, he was recognized as an authoritative microbiologist and headed a unique department at New York University.

It was singularly fortunate that he was Director of the Office of Science and Technology at the White House under President Lyndon Johnson when the U.S.-Japan CMSP was conceived. Although he received many honors, Dr. MacLeod thrived on sheer hard work, particularly in the fields of applied epidemiology and preventive medicine. His career was simply one of great service to man, and the U.S.-Japan CMSP has provided a striking example of his leadership qualities, a record of considerable achievement.

JAPANESE DELEGATION CHAIRMEN

Dr. Toshio Kurokawa

Dr. Toshio Kurokawa, one of the most distinguished clinical oncologists and researchers in Japan, earned a worldwide reputation in cancer research and control. In 1965, when the U.S.-Japan Cooperative Medical Science Program was initiated, he was appointed as Chairman of the Japanese Delegation, and in cooperation with his good counterpart, Dr. Colin MacLeod, Chairman of the U.S. Delegation, worked hard to organize the Program in a most appropriate way for both countries.

His outstanding leadership with deep insight, extensive knowledge, and warm personality were highly esteemed by all members of the Joint Committee of the Program. During his 14 years of service as Chairman of the Japanese Delegation from its creation in 1965 until 1979, he made several outstanding contributions to expand and develop the Program.

After his retirement from the chairmanship of the Japanese Delegation, he continued his activities serving as Honorary Director of the Cancer Institute Hospital of the Japanese Foundation for Cancer Research, and as a member of the Japan Academy, of which he became president in 1986. It is our great regret that Dr. Kurokawa passed away on February 21, 1988, due to a sudden heart attack, and that his long glorious career ended. On March 3, 1988, a funeral ceremony for Dr. Kurokawa was held at the Honganji Temple, Tsukiji, Tokyo, with a large number of his relatives, friends, patients, and others relating to his various activities, in attendance. All mourned the passing away of a great personality who had devoted his whole life to the advancement of medicine and the relief of patient suffering.

Profiles—Continued

Dr. Ivan L. Bennett, Jr.

Dr. Ivan Bennett has one of the most incisive minds in American medicine. Moreover, he has the uncanny facility to apply this incisiveness in a way that carefully separates the important from the unimportant. These character traits, and so many others, are responsible for the obvious fact that the U.S. Delegation of the U.S.-Japan CMSP has had only two chairmen, Dr. Bennett and Dr. MacLeod. They are similar clones.

Dr. Bennett was schooled as a clinician at Emory University and soon found additional footing in infectious diseases at Yale, Johns Hopkins, and, ultimately, New York University. Never one to follow, he has performed always as a leader whose associates and mentors rank him at the top of American medicine. He is a confirmed professional who walks with equal confidence in academic medicine and through the maze of Washington bureaucracy. Never is he ruffled by bureaucratic confusion or shallow thinking. He capably brushes the unimportant aside and has the special ability to bring an issue to its nearest conclusion and, in the process, to ensure that everyone involved is satisfied. The U.S.-Japan CMSP and its success are a tribute, in no small way, to Dr. Bennett's leadership and wisdom.

Dr. Norio Suwa

Dr. Norio Suwa is known among Japanese pathologists as a man of pride who is noble in appearance as well as in mind. His logical way of thinking and expressing himself is quite convincing, making it very difficult to refute him. During the severe period of campus conflicts in the late 1960's, when he was the Dean of Tohoku University Medical School, he faced the students straightaway calming them down and ending their riots.

After graduating from the Tokyo University School of Medicine in 1939, Dr. Suwa joined the Department of Pathology of that university. However, probably due to his frontier spirit and wish for independence, he soon moved from the university and became the Chief Pathologist of Komagome Hospital, a special medical center for infectious diseases of the Tokyo metropolis. Later, he returned to an academic position, becoming Chairman of the Pathology Department of Tohoku University School of Medicine, one of the seven "Imperial" universities of Japan. He produced many excellent research publications during his academic service, and the establishment of stereopathology should be pointed out as one of his most eminent contributions. At the age of 63, he retired from the university and ended his glorious career of academism.

Succeeding Dr. Kurokawa, Dr. Suwa was appointed as the second chairman of the Japanese Delegation and worked hard to further expand the Program. His great contributions to the Program are highly appreciated, and as a result of his pleasant personality and excellent ability with foreign languages, he is respected and beloved by all the members of both delegations.

Profiles—Continued

Dr. Shiro Someya

Dr. Shiro Someya started his research career at the Institute of Public Health in 1940 as a microbiologist. His major concern in the early days of his career was tuberculosis, and his research embraced not only problems of bacteriology and immunology but also the epidemiology of tuberculosis. In 1951, he was promoted to the Directorship of the Department of Microbiology of the Institute, and his research interests broadened to microbiology and immunology in general, and vaccine development. He was a creative investigator, and at the same time, he functioned both as an eminent mentor for young researchers and as an excellent administrator of the Institute. In 1965, he was promoted to the Vice Presidency of the Institute and, in 1972, to the Presidency. He retired in 1981. By virtue of his practical viewpoint regarding medical science in general, he became an indispensable member of the Japanese Tuberculosis Panel beginning with its debut in 1965. Since his nomination as a member of the Japanese Delegation in 1977, and particularly after he became Chairman of the Japanese Delegation in 1987, discussions in the Joint Committee meetings have become much more intense and objective. The U.S.-Japan CMSP now faces a new era of cooperation between the two countries that requires strong and understanding leadership as essential attributes for success. The members of both Delegations feel confident and secure with the excellent performance of Dr. Someya and will continue to provide to him their full and unstinted support.

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Highlights of the First 25 Years

During its 25 years of scientific research, the United States-Japan panel programs have contributed importantly to the improvement of health throughout the world. The following roster of important original discoveries *highlights* the impact of this cooperative program on the noble cause of public health and, in no way, dims the significance of so many valuable studies. Details of these scientific accomplishments are described in the individual panel sections.

AIDS PANELS

- Identification of intracellular regulatory elements which are responsible for HIV gene expression and viral regulation.
- Demonstration of genetic relationship between primate (SIV) and human (HIV) immunodeficiency viruses.
- Demonstration of similarities in latency and target organs for HTLV1 and HIV infections.

CHOLERA AND RELATED DIARRHEAL DISEASES PANELS

- Development of a single oral rehydration solution which is universally effective in treatment of diarrhea caused by both *V. cholera* and *E. coli*.
- Biochemical and immunological characterization of *V. cholerae* enterotoxin, demonstration of its three dimensional structure, and cloning of the gene which encodes it.
- Biochemical and immunological characterization of the two major *E. coli* enterotoxins, and demonstration of the plasmid genes by which they are encoded.

ENVIRONMENTAL CARCINOGENESIS AND MUTATGENESIS PANELS

- Development, refinement, validation, and application of short term tests for detection and characterization of mutagens, carcinogens and tumor promoters in the environment.
- Identification and characterization of natural products, chemicals formed by normal cooking methods and synthetic chemicals as mutagens and carcinogens.
- Characterization of oncogene activation in chemically induced and spontaneous tumors in humans and experimental animals.
- Development of methods to use specific, sensitive, molecular indicators to monitor and quantitate the exposure of various human populations to environmental mutagens and carcinogens.

HEPATITIS PANELS

- Immunoprophylaxis for prevention of perinatal transmission of hepatitis B (HBV) infection.
- Demonstration of the relationship between hepatitis B (HBV) and C (HCV) infection and carcinoma of the liver.
- Molecular characterization, cloning and sequencing of the genomes of hepatitis A virus (HAV), HBV, HCV, and cloning and sequencing of the genomes of hepatitis D virus (HDV) and hepatitis E virus (HEV).

IMMUNOLOGY BOARDS

- Discovery and characterization of new cytokines, including interleukin-6, that affect B lymphocyte differentiation, selective gene activation, and immunoglobulin isotype expression.
- Identification and characterization of T-cell receptors, evaluation of genetic control of T-cell receptor specificity, and of the molecular basis of T-cell selection in the thymus.
- Use of transgenic mice to determine the physiologic effects of cytokines, including interleukin-2.
- Provision of critical collaborative support to the Panels in their biomedical research programs.

LEPROSY PANELS

- Development of experimental models, namely, the mouse foot pad and the armadillo for cultivation of *M. leprae*, that are useful for vaccine and drug evaluation. This led to large scale human trials of multiple drug regimens and vaccines.
- Identification, through application of molecular genetic techniques, of the important cell wall antigens of *M. leprae*, and development of monoclonal antibodies to them.

MALNUTRITION PANELS

- Determination that the protein sparing effect of dietary energy appears to be more important than the protein intake itself.
- Demonstration that the consumption of vitamin C and the production of epinephrine is increased by stress.
- Establishment of the role of vitamin A and iron and calcium deficiency in Southeast Asia which led to measures aimed at reducing the prevalence of these conditions.

PARASITIC DISEASES PANELS

- Demonstration of protective immunity in primates against *Schistosoma japonicum* by exposure to homologous, irradiated cercariae.
- Demonstration that idiotypic and anti-idiotypic regulation are both important at given times after murine infection with *S. japonicum*.
- Identification of the family of sialoglycoproteins known as glycophorins as the major erythrocyte receptor for *Plasmodium falciparum*.

TUBERCULOSIS

- Clarification of the mechanism of cellular immunity and the significance of adjuvant activity of the tubercle bacillus.
- Fractionation and identification of the biologically and immunologically active constituents of *M. tuberculosis*.

VIRAL DISEASES PANELS

- Development of a new rabies vaccine.
- Significant clarification of the pathogenesis and basic immunology of the hemorrhagic fevers.
- Broad expansion of the knowledge of viral gastroenteritis.

Dr. Ivan Bennett chairs the 25th Meeting of the Joint Committee in Bethesda, Maryland, with Dr. Shiro Someya.

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JOINT COMMUNIQUE
PRESIDENT JOHNSON AND PRIME MINISTER SATO
JANUARY 12 and 13, 1965

President Johnson and Prime Minister Sato met in Washington on January 12 and 13, 1965, to exchange views on the current international situation and matters of mutual interest to the United States and Japan. They were assisted by Secretary Rusk and Foreign Minister Shiina and Secretary-General Miki of the Liberal Democratic Party.

The President and Prime Minister reviewed the present international situation and reaffirmed the partnership of the two countries which grows out of common beliefs and the shared objective of a lasting peace based on justice, freedom and prosperity for all people. They expressed a firm determination that the two countries should cooperate more closely in seeking this common objective. They agreed that for this purpose the two countries should maintain the closest contact and consultation not only on problems lying between them but on problems affecting Asia and the world in general.

The President and the Prime Minister, recognizing the valuable role of the United Nations in the maintenance of the peace and prosperity of the world, exchanged frank views on the difficult questions now confronting the United Nations, and agreed to continued cooperative efforts to strengthen the functions of the United Nations and to enhance its authority.

. . .

The President and the Prime Minister, mindful of the many areas of human health which are of great concern to all the peoples of Asia, agreed to undertake a greatly expanded program of cooperation in medical science with respect to such diseases as malaria, cholera, schistosomiasis, tuberculosis, and stomach cancer, in addition to cooperative efforts on problems of air pollution and pesticides. As a first step to implement the agreement, they agreed to convene a conference of the foremost medical scientists from the United States and Japan to work out the details of the new program for discussion with other governments concerned.

The President and the Prime Minister expressed their satisfaction with the meeting just held and their desire to continue to maintain close personal contact.

THE 24TH MEETING OF JAPAN-UNITED STATES
COOPERATIVE MEDICAL SCIENCE PROGRAM
JULY 21-22 1988

The 24th Meeting of the Joint Committee was held in Tokyo in 1988.

Dr. Theodore Woodward, a charter member of the Program, and Dr. Earl Beck, NIH Secretariat, at the 1986 meeting held in Toyama, Japan.

Dr. Colin MacLeod and Dr. Toshio Kurokawa, Chairmen of the U.S. and Japanese Delegations confer during the First Joint Committee Meeting in Honolulu, 1965.

Japanese and American members of the Subcommittee on Program Review and Planning meet in Kona, Hawaii, in 1989.

第22回 日米医学協力委員会

Members of the Delegations at the 22nd Joint Committee Meeting in Toyama, Japan.

Dr. David Rall (left), Dr. Donald Whedon, Dr. Robert Shope, and Dr. Charles Carpenter of the U.S. Delegation during the Joint Committee Meeting in Toyama, Japan, 1986.

Dr. Tadao Shimao and Dr. Takashi Sugimura confer during the 1989 Joint Committee Meeting in Bethesda, Maryland.

Members of the Delegations relax in the Blue Ridge Mountains following a visit to the University of Virginia School of Medicine.

Dr. Toshitsugu Oda, Dr. Akira Oya, and Dr. Tadao Shimao enjoy the hospitality of the Woodwards, 1989.

Two boatloads of Delegates head for Parson's Island, Maryland, as guests of Dr. and Mrs. Woodward following the 1989 Joint Committee Meeting.

INTRODUCTION

This is the fifth in a series of reports for the U.S.-Japan Cooperative Medical Science Program (U.S.-Japan CMSP). These reports cover 5-year periods of Program events and scientific progress. The First Five-Year Report, 1965–1970, was adopted by the Joint Committee at its sixth meeting. The Second Five-Year Report, 1970–1975, was accepted by the Joint Committee at its 11th meeting. That publication was dedicated to Dr. Colin MacLeod, Chairman of the U.S. Delegation and Co-Chairman of the Joint Committee from 1965 until his death in 1972. The Third Five-Year Report, 1975–1980, was adopted by the Joint Committee at its 16th meeting. It was dedicated to Dr. Toshio Kurokawa, Chairman of the Japanese Delegation and Co-Chairman of the Joint Committee from 1965 until his retirement in 1979. The Fourth Five-Year Report, 1980–1985, was dedicated to Dr. Ivan L. Bennett, Jr., Chairman of the U.S. Delegation and Co-Chairman of the Joint Committee, and to Dr. Theodore E. Woodward, member of the Delegation and of the Joint Committee since its inception in 1965. The present report, encompassing the 5-year period between 1985 and 1990, is dedicated to Dr. Norio Suwa, Chairman of the Japanese Delegation from 1979 to 1988.

Preparations for the Fifth Five-Year Report began at the Subcommittee on Planning and Program Review Meeting in February 1989. At the Joint Committee Meeting, in July 1989, final plans were adopted for publication of the document in July 1990. This fifth report marks a quarter-of-a-century of noteworthy scientific progress between the two nations. Of special significance is that the program has adopted state-of-the-art technology and there has been a continuing expansion of the use of molecular biology and recombinant DNA technology in the research conducted by both the Japanese and American Panels.

In their planning sessions, the Joint Committee agreed that the same general format used for previous reports be adopted and that the Japanese Delegation accept the responsibility for designing the cover, a responsibility that alternates between the two countries. The Joint Committee accepted the recommendation of the Subcommittee that the theme of the report would be "Significant Advances Produced by the Program Over the Past 25 Years."

ORGANIZATION AND HISTORY

In January 1965, the President of the United States and the Prime Minister of Japan met in Washington, D.C., to discuss many important matters concerning the two nations. It was during this meeting that the two heads of state agreed to undertake a greatly expanded, joint cooperative research effort in biomedical sciences, concentrating on health problems of recognized importance in Asia. The new bilateral program was named the United States-Japan Cooperative Medical Science Program (U.S.-Japan CMSP).

Medical advisers from the respective countries met in April and again in October of 1965, selecting cholera, leprosy, parasitic diseases, tuberculosis, and viral diseases as initial areas for collaborative research. In 1966, research on malnutrition was added, and 6 years later, in 1972, the Program was broadened to include investigations on environmental mutagenesis and carcinogenesis. In 1979, research on viral hepatitis, a disease of worldwide importance, was added, expanding the Program from seven to eight disease areas. This was not to be the last addition, because the Program continued to gain momentum and grow. In 1981, a decision was made to include immunology as a new research area, since it had direct application to most, if not all, the other eight areas.

The Program was defined to operate within a bilateral governmental framework which could involve scientists and facilities in Third World countries in collaborative efforts of mutual interest. Cooperation with the World Health Organization (WHO) has always been considered appropriate and has been encouraged.

The relevant regions in Asia are not specifically delineated but are generally understood to include the Republic of Korea in the north, India and Pakistan to the west, and other intervening nations in the broad Pacific Basin.

The United States and Japan each support the costs of its own scientific projects and meetings. Research by U.S. scientists is funded by the U.S. Government and that of Japanese scientists by the Japanese Government.

The Joint Committee is expected to advise its respective governments on the scope, direction, and other broad aspects of the program and to develop review procedures necessary to assure fulfillment of the objectives for which the Program was established.

The panels have achieved significant progress in their respective areas of research. To keep the research focused, each panel developed specific guidelines which have been appropriately refined and modified as research has progressed. The results of the four previous publications are briefly summarized in this report.

During the fifth 5 years of the Program, emphasis on collaboration between scientists of the two countries resulted in increased participation and open discussion at the annual conferences and an enhancement of scientific progress. The most significant event was the recognition of the importance of Acquired Immunodeficiency Syndrome as a devastating disease that is spreading throughout the world. In view of this, the Joint Committee initiated the formation of Joint AIDS Panels and the first Joint AIDS Conference was held in 1988.

ACTIVITIES OF THE JOINT COMMITTEE

During the fifth 5-year period, Joint Committee meetings continued to be held annually, alternating between Japan and the United States. Meetings of the Joint Subcommittee on Program Review and Planning were held in Hawaii each February and again just prior to the Joint Committee meetings.

In 1985, the 21st Joint Committee meeting was held in Washington, D.C., in the Loy Henderson Conference Room, Department of State. A Fourth Five-Year Report, 1980–1985, describing the scientific progress and administrative activities of the U.S.-Japan Program, was adopted and dedicated to Dr. Ivan L. Bennett, Jr., the Chairman of the U.S. Delegation and Dr. Theodore E. Woodward, who has been a member of the U.S. Delegation since the inception of the Program. Both were present to receive this special tribute from their colleagues. The Report not only described, in some detail, the research activities of the Panels over the preceding 5 years but briefly reviewed significant accomplishments of the Program over the two decades of its existence.

The emerging threat of HTLV-III infection was discussed, and it was suggested that the Joint Hepatitis Panels consider the inclusion of this disease in their guidelines.

Prior to the Joint Committee meeting, a comprehensive Symposium on Malnutrition was held at the National Institutes of Health on July 16–18. The symposium was organized along Program guidelines and served to sharpen and redirect the research activities of the Joint Malnutrition Panels. The symposium also served to initiate a year of formal review of the Panels' activities.

The 22d Joint Committee Meeting was held July 24–25, 1986, at the Toyama Kenmin Kaikan, Toyama City, Japan. This meeting, being held outside of Tokyo, was organized by Dr. Manabu Sasa and endorsed by the Honorable Yutaka Nakaoki, Governor of Toyama Prefecture. As customary, the Chairmen, or their representatives, of the Panels/Boards presented summarizations of the previous year's research activities. Immediately preceding the Joint Committee Meeting, the Joint Cholera Panels and Joint Immunology Boards held their annual conferences, in Toyama, summaries of which were presented to the Joint Committee. Stimulating progress was reported in the control of cholera using inactivated and genetically engineered live attenuated vaccines. The Immunology Boards focused on new immunologic concepts and the rapidly improving technical procedures that will not only assist the Board in reaching its goals but will also assist the Panels in pursuit of their work. Toward this end, the Immunology Boards will meet jointly with the Panels in future years.

In the Five-Year Review of the Malnutrition Panels it was pointed out that the guidelines had been revised and that the scientific programs were better coordinated and conducive to future collaborative research.

The 23d Joint Committee Meeting was held July 23–24, 1987, at the National Institutes of Health in Bethesda, Maryland. The highlight of the meeting was an address by Dr. Anthony S. Fauci, Director of the National Institute of Allergy and Infectious Diseases and member of the U.S. Delegation. Dr. Fauci presented an illuminating review of the major aspects of Acquired Immunodeficiency Syndrome (AIDS) that was followed by discussion of proposed guidelines for new Panels on AIDS. After acceptance of the guidelines, the Joint Committee unanimously recommended that Joint Panels on AIDS be organized as soon as possible.

After hearing the reports of the Joint Panels/Boards, the Committee was convinced that research progress had been sparked by the application of new technology to the solution of old problems. The Committee was likewise pleased by the progress being made by the Cholera Panels and the Immunology Boards as indicated in their Five-Year Reviews. In view of the significance of diarrheal diseases other than cholera, research by the panels including new vaccine development has been extended to other enteropathogenic bacterial diarrheas. To better indicate the scope of the panels' activities, the Joint Committee approved a change in the name to "Cholera and Related Diarrheal Diseases Panel." The 5 years of activity by the Immunology Boards confirmed the importance of unraveling the complexities of the immune system and its intimate involvement in the wide variety of human diseases covered by the Panels. To promote interaction of the Boards with the Panels, the Boards planned to meet with the Joint Viral Diseases Panels at their next meeting to share information and evaluate new approaches to the problems of viral diseases.

The 24th Annual Meeting of the Joint Committee was held at the Ministry of Health and Welfare in Tokyo, Japan, on July 22 and 23, 1988.

On July 18–20, preceding the Joint Committee Meeting, Symposia on Parasitic and Viral Diseases were held by the respective Joint Panels in preparation for their 5-year reviews. These symposia were particularly impressive with spirited discussion and innovative suggestions for the future.

Reports of the individual panels' activities were presented to the Joint Committee by Panel Chairmen or their representatives. Included in these reports were brief reviews of the scientific progress made during the past year as well as comments regarding the significance of the work. The Committee expressed satisfaction with the continued progress sparked significantly by the application of newer technology to the solution of older problems and particularly the helpful impact and advice given by the Immunology Board to all Panels.

The Five-Year Review Reports on Leprosy and Tuberculosis were presented to the Joint Committee. The Leprosy Panels had made much progress during the years covered by the report. They adapted to new research opportunities and were using the newest and most modern technical procedures available. The Joint Committee was impressed with the scientific work of these panels and recommended that their program activities be continued. The reviewers of the Tuberculosis Panels submitted a very complimentary report stating that the Joint Panels had vigorously pursued their guidelines and had effectively adapted their research activities to the available new technology. The Joint Subcommittee concurred with the recommendation that tuberculosis continue as a separate program and that the Leprosy and Tuberculosis Panels meet jointly because of their common interests.

The Joint Committee held its 25th Annual Meeting at the National Institutes of Health in Bethesda, Maryland on July 20 and 21, 1989. At this meeting it was announced that the Program would celebrate its 25th Anniversary in 1990 and a special program was being prepared for the Joint Committee meeting in Tokyo in July.

Brief reviews of the scientific progress made during the past year as well as comments regarding the significance of the work were presented to the Committee members who expressed satisfaction with the continued research progress and the continuing application of new technology to the solution of old problems.

In December 1988, the Japanese AIDS Panel hosted the first Joint Conference of the AIDS Panels at the National Cancer Institute in Tokyo, to discuss the epidemiology, virology, and immunology of HIV infections and to define ways in which both countries might cooperate to contain infection. Some 5–10 million persons worldwide are currently infected, and it was predicted that in 1992 AIDS will be the leading cause of death in the United States. It is difficult to estimate the full human dimension of the disease and the tragedy to the unborn from perinatal infection, a rapidly increasing mode of transmission in the inner city in IV drug users and their sexual partners. The situation in Japan stands in welcome relief where the few hundred cases expected in the next few years will be limited by their well-organized surveillance system and rapid response to contain HIV infections derived from experience elsewhere."

The Five-Year Review Reports on the Parasitic Diseases and Viral Diseases were presented to the Joint Committee. It was reported that the symposium, held in 1983, had a profound effect on the Parasitic Diseases Panels resulting in the scope of study and level of competence had greatly increased. Work was described as being at the frontier of molecular biology of parasites. In addition, the previously recognized lack of focus of the Viral Diseases Panels had been corrected and commented on the progress evidenced by a new rotavirus vaccine, a novel approach to rabies vaccine with a vaccinia vector, and similar work with Japanese encephalitis. The Joint Committee accepted the recommendations and commended the Panels for their excellent work.

Plans were made for organizing and publishing the Fifth Five-Year Report, 1985–1990. This report will represent 25 years of scientific achievement facilitated by collaborative efforts of the scientists of the U.S.–Japan Cooperative Medical Science Program.

GUIDELINES

An extensive review of the guidelines set forth for the original Panels, those approved for new Panels/Board, and changes occurring over the first 20 years of the Program was published in the Fourth Five-Year Report. Here, only changes in guidelines made during the last 5-year period are presented. The current guidelines are included in the individual Panels/Boards sections of this report.

In July 1990, the Joint Committee adopted new guidelines for the Joint Malnutrition Panels. The previous and new guidelines are listed below.

JOINT PANELS ON MALNUTRITION
United States-Japan Cooperative
Medical Science Program

PREVIOUS

The malnutrition program has the following five research objectives.

1. Influence of environmental and host factors on requirement for health in Asian countries.
2. Studies of the health significance of iron deficiency and methods for its eradication.
3. Investigation of the interactions of nutrition and infection with special emphasis on the mechanisms responsible.
4. Determination of the effects of nutrition on physical and mental development, behavior, physical capability, and work performance.
5. Health consequences of different (and changing) dietary patterns and food habits.

These guidelines are under active revision by the Joint Panels at the time of publication of this Report.

JOINT PANELS ON MALNUTRITION
United States-Japan Cooperative
Medical Science Program

NEW

It is recognized that nutrition-related disorders are serious problems in Asia as well as other parts of the world. During a review of the U.S.-Japan Joint Malnutrition Panels, there was a gradual, but perceptible, shift in emphasis to increasing attention to nutritional concerns of the United States and Japan, which will subsequently impact on the nutritional problems of other countries in Asia. Of particular concern is the health consequences of different and changing dietary patterns and food habits. Dietary patterns have been recognized as a contributing variable or risk factor for a number of chronic diseases. With the current influences of the Westernized diet on food habits in Japan, evidence relating dietary components to chronic disease through basic, clinical, and epidemiological investigations would be of great importance for both countries. To accomplish this task, it would be of value to have improved measures of nutritional assessment which may serve as prognostic indicators of the health consequences (both positive and negative) of certain dietary habits. The following areas have been identified for developing a collaborative research program.

1. Health consequences of changing dietary patterns on health, development of disease, and disease prevention.
    - A. Fatty acids metabolism, including unsaturated fatty acids, in diet and health
    - B. Bone disease in relation to diet
    - C. Effect of sulfur amino acids
2. Nutritional assessment
3. Endogenous mediators of nutritional metabolism, not limited to mediators generated by inflammation and infection, but also mediators associated with other types of diseases, such as malignancies.

7/26/90

The guidelines for the Immunology Boards were revised in July 1987, with the addition of a research area for molecular genetics. The previous and the new guidelines are presented below.

JOINT IMMUNOLOGY BOARDS
United States-Japan Cooperative
Medical Science Program

PREVIOUS

Immunology has made rapid advances in the last decade. The recent introduction of the techniques of somatic cell hybridization, molecular genetics, and long-term culture of immunocompetent cells to the study of the immune system has caused an unprecedented increase in our understanding of this field. Such progress in fundamental immunology has the potential to revolutionize the diagnosis, treatment, and prevention of immunologic and infectious diseases, including those being studied under the auspices of the U.S.-Japan Cooperative Medical Science Program.

A long-term objective of the Immunology Board is to study and elucidate the cellular and molecular mechanisms of the immune response and to define effective approaches for the manipulation of the immune system.

In order to achieve these purposes, the Board will focus its concern on the following research areas:

1. Molecular studies of the genetic organization of the lymphoid system.
2. Cellular and molecular studies of lymphocyte activation, proliferation, and differentiation.
3. Immunogenetic approaches to the study of lymphocyte interactions, with emphasis on the role of histocompatibility gene products in the regulation of the immune response.
4. Cellular and molecular studies of the regulation of the immune response, with emphasis on the role of histocompatibility gene products in the regulation of the immune response.
5. Application of techniques for the development of stable cell lines and of somatic cell hybrids to the study of the regulation of lymphocyte activation and function and to the preparation of monoclonal antibodies, lymphocyte receptors, and specific, as well as nonspecific lymphocyte products.
6. Mechanisms to support the interaction of immunologists and the exchange of information and material between the two countries.

JOINT IMMUNOLOGY BOARDS
United States-Japan Cooperative Medical Science Program

NEW

The original guidelines remained in effect until July 23, 1987, when another research area was added as follows:

1. Application of molecular genetics to study functions of the immune system, including genes encoding cell surface antigens, secreted immunoregulatory factors, and important foreign antigens.

7/23/87

The Joint Cholera Panels guidelines have been changed repeatedly during the first 20 years of the Program. Since the name Cholera Panel did not accurately describe the areas of research being studied under the guidelines, the Joint Committee adopted the recommendation of the Subcommittee on Program Review and Planning to change the name to the Cholera and Related Diarrheal Diseases Panel and to retain the current guidelines.

REVIEW OF PANEL ACTIVITIES

The plan for Program reviews specifies that the Co-Chairmen of the Joint Committee appoint a subcommittee comprising of members of each delegation to review and to evaluate the accomplishments of existing panels and boards with respect to the overall goals of the U.S.-Japan CMSP. Primary attention is directed toward research guidelines that have been identified by the panels/board and approved by the Joint Committee. Criteria regarded as essential for a proper evaluation of the respective guidelines as they relate to the current research programs are:

1. Does the research outlined in the guidelines remain relevant to the objectives of the U.S.-Japan CMSP?
2. How well does the scientific program fulfill its research guidelines? Is the research program sufficiently concise to ensure achievement of its designated basic and practical objectives, and does it avoid diffuseness which could lead to ineffectiveness?
3. How appropriate are these guidelines in the light of new and current knowledge, needs, and opportunities?
4. Realistically, in terms of scientific applications, can these objectives be achieved by pursuing further the approaches currently being utilized?
5. Is there a reasonably identifiable timeframe wherein the research objectives of the guidelines can or should be reached, modified, or terminated?

It is understood that the respective U.S. and Japanese reviewers will prepare a combined report carefully evaluating the progress of the panel or board under review in accordance with the five review criteria. It is the prerogative of the reviewers to invite outside consultants to assist in the evaluation process if deemed advisable. The review report is submitted initially to the Subcommittee on Program Review and Planning for comment and approval before being presented to the Joint Committee for a final decision. This decision is based on the following:

When compared with other potential program areas which also may offer promise for progress and accomplishment, should the panels/board under review be:

a. modified
b. combined with another program area
c. reduced or expanded in scope
d. recommended for other sponsorship
e. phased-out in an orderly manner
f. continued as currently programmed?

Each panel/board review is arranged in accordance with the following format: introduction, list of present and past members, summary of research progress under each guideline, and a concise evaluation of whether the research is responsive to the five criteria previously cited.

In this Report only a portion of the review for each panel is presented.

PANEL REVIEWS

Malnutrition Panels

Reviewers: Dr. Hidio Fukumi
Dr. Donald Whedon

1. The research fostered and supported by the Joint Malnutrition Panels was summarized in the progress report pub-

lished in the Five-Year Report just before the review of these Panels. In that progress report, it is clear that the research remains relevant to the objectives of exploring scientific measures to deal with the health problems of Asia. At the same time there has been a gradual shift in the research projects toward attempting to deal also with problems of considerable interest and concern in Japan and the United States.

2. The Panels' scientific program as described in the Five-Year Report is, on both sides, well within the areas of major interest (guidelines) which have been directive over at least the past 5 years, most for much longer. The projects themselves have in most instances been aimed at solving specific scientific questions; they have had to be so or they would not have passed scientific merit and relevance reviews for funding. The success of the projects' continuing merit and relevance may be attested to by a statistic from the U.S. program; for the 13 projects in FY 1983 which were beyond the 3d year (that is, in a phase of support renewal), the average "age" was 8.2 years, indicating that most had successfully competed for at least a second renewal. A problem of diffuseness versus conciseness lies in the guidelines or objectives as noted below.

3. From time to time, the stated objectives or guidelines have been commented upon as being unusually broad. To speak favorably of them, they apparently have been sufficiently specific to produce stimulation of many research projects, but on the other hand, present review and reconsideration of them by the Malnutrition Panels was clearly in order "in the light of new and current knowledge, needs, and opportunities." Revision, remodeling, or rejection of the guidelines in place for several years is much in order, and the discussions taking place in 1984 and 1985 have resulted in major changes. This is a favorable development and, we trust, symptomatic of rejuvenation and to-be-anticipated continuing health of the Program in both Japan and the United States.

4. On the U.S. side during the early years of the Program, it received "nutrition for growth and development" from its fostering National Institute of Arthritis and Metabolic Diseases (NIAMD), including a "special study section" for review of proposed projects. At the urging of the NIAID, the parent of all of the other panels, NIAMD reluctantly conformed to the mode of review of the NIAID, i.e. regular Division of Research Grants study section review, a sensible system in an institute (NIAID) where U.S.-Japan projects are a significant part of the Institute's total program. Since that change, a periodic plea by the Panel Chairman for "programmatic assistance" has continued to this day in an atmosphere where the major overall concern has been for individually initiated research grants judged by peer review. If the question can be honestly raised that "malnutrition" applications do not receive true peer review in the regular study sections, then an opportunity exists for two National Advisory Councils and two new Institute Directors to consider some "imaginative programming." Realistically, it will probably take strong evidence to make a convincing case that the Panels' objectives cannot, in the United States, be achieved by pursuing further the approaches currently utilized by the Panels.

5. The problems of malnutrition remain sufficiently complex, difficult, and widely distributed around the world that it is impossible to expect that the Panels' objectives can be reached at any early time. As has been pointed out, since malnutrition is interwoven with the disease problems confronted by the other panels in the Program (which involve, in various ways, infection and the immune response to infection), the relevance and importance of the objectives and activities of the malnutrition program remain high.

We believe that significant contributions have been made in the past and are continuing to be made by the Malnutrition Panels' activities in dealing with nutritional problems in Asia and, more recently, relative to other problems in Japan and the United States. With revised, up-to-date guidelines and with anticipation of more in the way of bilateral research collaboration, we are indeed expectant that additional substantial contributions will be made with the continuation of this program.

Viral Diseases Panels

Reviewers: Dr. Charles Carpenter, Jr.
Dr. Yuichi Yamamura

1. The activities of the Viral Diseases Panel have closely followed the guidelines. Thus, their research remains entirely relevant in that they are applying the tools of modern molecular biology to the solution of major health problems of Asia. Since the last review of the activities of the Viral Diseases Panels, major strides have been made in combating the worldwide morbidity and mortality caused by viral diseases. Major progress has been made in the fields of viral gastroenteritis, rabies, and arbovirus diseases.

One of the major efforts has been directed toward development of a vaccine against rotavirus, which is now known to be one of the two leading killers of children under the age of 2. Initial field trials of live reassortment vaccines, containing genes for all four known retroviral group A serotypes, have been promising.

A unique new approach to a rabies vaccine utilizes a vaccinia virus vector which has been genetically modified by transposition with a gene which encodes a rabies glycoprotein. When administered orally, it causes a protective immune response in captured raccoons. This may prove to be a major advance in view of the widespread penetration of rabies in the raccoon population in the Eastern United States.

In another novel approach murine CTL clones have been isolated from a/j mice immunized with HEP antigens. Those CTLs protected mice when transferred to mice treated by cyclophosphamide and infected with ERA strain of rabies virus through the plantar route. The CTLs lysed the cells infected with ERA strain of rabies virus.

Research on the hemorrhagic fever with renal syndrome (Korean hemorrhagic fever) has led to a far greater understanding of this hitherto poorly understood, but widely distributed and potentially fatal, viral illness.

Envelope glycoprotein E of Japanese encephalitis virus has been extensively studied. An epitope has been studied which stimulated antibodies that conferred very high protective activity against Japanese encephalitis (JE) virus infection in mice. This epitope was JE-specific and conserved among various strains of JE virus. By analyzing base sequences of an escaped mutant of this epitope, it was suggested that this epitope is located in the N-terminal side of E protein. E protein was only successfully expressed by recombinant vaccinia virus or baculovirus in which E gene was inserted with its upstream genes together.

Japanese workers found that a single amino acid change correlated with the subtype specificity in the JE virus neutralization test. They also analyzed nucleotide sequences of an attenuated JE virus used for swine vaccine and found four amino acid substitutions in E protein as compared with that of the parent strain.

2. The Panels' scientific programs have become increasingly well-focused as increasingly sophisticated techniques have been applied to delineating the pathogenetic processes by which viruses cause disease in the human host. This has permitted more precise approaches both to the treatment of viral diseases and to developing protective immunity against viral infections.

The Panels have thoughtfully moved from the original emphasis on descriptive epidemiology, to apply the tools of modern molecular biology to major viral diseases which are epidemic and endemic in Asia.

They have succeeded in developing a candidate live virus vaccine against rotavirus, a major pathogen which had not yet been identified at the inception of the U.S.-Japan Cooperative Medical Science Program.

They have recruited outstanding scientists in both nations to study the virus responsible for Korean hemorrhagic fever; they have defined the rodent host and the wide geographic extent of this illness, and are applying sophisticated technology to define the pathogenic mechanisms and immunologic responses to this disease.

They have improved the approaches to immunization against rabies and are attempting to determine the optimal approach to immunization against dengue fever.

3. The Viral Diseases Panels have altered and extended their objectives as new major viral problems have arisen and new basic knowledge of the molecular biology of viruses has arisen. As the major role of viral diarrheas in childhood mortality in the developing world was clearly established, viral gastroenteritis became a priority research area for the Viral Disease Panels. Tens of millions of children are afflicted every year and over one million die from severe diarrhea. Research on rotaviral diseases is focused on the proteins produced by the virus, the biological function of these proteins in pathogenesis, and the host's immune responses. There is also considerable research interest on differentiating various isolates and strains of RV by their antigenic structure, and a concerted effort is being directed toward vaccine development and evaluation.

Likewise as the worldwide prevalence of the hemorrhagic fever with renal syndrome was clarified, this viral illness was accorded high priority by the Virus Panels. Research on Hantaan virus (HV) and hemorrhagic fever with renal syndrome (HFRS) conducted by scientists from the United States has emphasized the comparative molecular genetics of the viral genome and epidemiology of the disease. A clinical trial with the antiviral drug, ribavirin, has been conducted in an endemic area of China with negative results. Several genetically distinct but serologically related hantaviruses have been isolated from different geographic areas within and outside of the United States. U.S. scientists are also exploring the potential of vaccines through recombinant DNA techniques.

Progress of research on HFRS is reported regularly at the annual joint meetings of the U.S. and Japanese Panels. As with rotaviruses, U.S. and Japanese collaborations are accelerating the pace of research on hantaviruses and HFRS. Effective collaborations have developed from the congenial atmosphere generated by the joint annual panel meetings. These collaborations are especially important in view of the small number of scientists whose research is focused on this field.

Overall, the Viral Diseases Panels have done an excellent job of focusing on those viral diseases which pose a major thrust to Asia and the Pacific Basin, and are effectively applying the tools of modern molecular biology to their solutions.

4. In terms of scientific applications, it is reasonable to predict that the objectives of the Viral Diseases Panels can be achieved by further pursuit of the approaches currently being utilized. The approaches are being appropriately modified as the science of molecular biology advances, and it is entirely realistic to expect that the flexibility and adaptation of the panels to new technology will continue to be strong points in their work.

5. The identification of a timeframe or point in time where the objectives can be reached would be very difficult. The problems presented by the viral diseases which are being approached by the Panels are sufficiently complex that it is impossible to predict the timeframe within which the Panels objectives will be achieved. Different approaches must be taken for the control of each viral pathogen. The approach to vaccine development which has proved so successful with rabies, for example, is not applicable to the problem of Japanese B encephalitis.

Therefore, although it can be predicted with great assurance that the current approaches of the Viral Diseases Panels will be effective in reducing the burden of viral disease throughout the developing world, it would be unrealistic to predict a timeframe within which each of the Panels' goals will be accomplished.

Parasitic Diseases Panels

Reviewers: Dr. Barry R. Bloom
Dr. Wataru Mori

1. The Parasitic Diseases Panels were established to support and encourage research in the area of parasitic diseases in 1966, with an almost exclusive focus on helminthic infections, particularly schistosomiasis and filariasis. Parasitic and tropical diseases remain the major burden to 500 million people in the developing countries of Asia and the Third World. The research of these Panels is crucial to understanding and treatment of these diseases. Helminthic infections represent a major problem of morbidity and mortality worldwide; the study of metazoan parasites and their vectors remains a very difficult and challenging scientific problem.

It is gratifying that significant contributions to the molecular biology of parasites have been made by scientists from both countries. Monoclonal antibodies have been prepared against major antigens of virtually every species of parasite studied. In the cases of schistosomiasis, filariasis, and malaria, genes encoding important antigens from each of the parasites have been cloned and many have already been sequenced. Indepth analysis of many of these antigens has revealed some of their biological functions as well. For example, in the case of schistosomes, a 26-28kDa antigen encodes the enzyme glutathione S-transferase, another 28kDa antigen has been found to be triose phosphate isomerase, a 97kDa myofibrillar protein encodes paramyosin and another recombinant antigen shares sequence homology to the mammalian myosin heavy chain. Several

S. mansoni antigens are proteases, with homology to cathepsin B and hemoglobinase, which may be involved both in parasite nutrition and in skin penetration by cercariae. Further, in the case of plasmodial antigens, evidence was provided indicating that several may serve as receptors for binding to the red cell, and the nature of the erythrocyte ligands is currently being elucidated, with clear evidence for involvement of certain glycophorins dependent on their state of O-acetylation of sialic acid. An extraordinary molecular biological discovery was a striking DNA sequence homology between the envelope glycoprotein sequence of murine retrovirus proviral DNA (*env*) and genomic DNA of *S. japonicum* and *S. mansoni*, as well as homology between gag and the eggs of *S. mansoni* and a portion of the pol sequence and adult forms of *S. japonicum*. Recombinant DNA fragments derived from the *S. mansoni* eggs did not hybridize with DNAs of male or female adult worms of *S. mansoni* suggesting rearrangements and deletions of DNA sequences occurs during development of *S. mansoni*.

Molecular approaches to drug design have similarly been taken. For example, the *P. falciparum* enzyme dihydrofolate reductase-thymidilate synthetase (DHFR-TS), the target of pyrimethamine action, has been cloned and mutants have been produced that exhibit significant gene duplications and translocations associated with altered enzyme production and specific activity. The carcinogenic metabolite, N-OH-TRP-P-2, of a promutagen was present in higher levels in the serum of schistosome-infected mice, suggesting that hepatic cells in schistosome-infected individuals might be concentrating a carcinogen that could possibly be related to the subsequent development of neoplasia in chronically infected individuals.

Highly sophisticated immunologic approaches have been developed by scientists in both countries to study immunoregulation in these diseases, which can only be enumerated here. ELISA assays for antigen and antibodies are being developed in both countries that may make important contributions to diagnostic and epidemiological tests that can be carried out under field conditions.

An IgE-producing hybridoma specific for an epitope on several schistosome antigens has been developed that should be highly useful in facilitating the understanding of the role of eosinophils in destruction of schistosomula and possibly production of fibrosis and other pathologic changes. In the case of experimental schistosomiasis, clear-cut evidence of idiotype/anti-idiotype interactions involving both antibodies and T cells, has been obtained. These results present encouraging possibilities of immunizing against carbohydrate epitopes using idiotypic antibodies in vaccination studies and of generating idiotype-specific suppressor cells that down-regulate granuloma formation in studies of pathogenesis. Elegant experiments on the genetics of immune responses in schistosome-infected human donors has revealed important evidence for the existence of genetic control of T-cell suppressive responses, implicating HLA-DQ as a restricting element in controlling T-cell suppressive responses in man.

High levels of protective immunity could be induced in mice against *B. malayi* by immunization with microfilariae emulsified in adjuvants in certain strains of mice, but not in others, indicating some genetic control of responses to this parasite.

T-cell lines and clones derived from individuals infected with a variety of parasites have been developed in both countries. These are being used for analysis of antigens and epitopes recognized by T cells, and elucidation of the role of cellular immune responses in restricting the growth of parasites and in producing pathological changes. In the case of *P. chabaudi*, there is striking evidence that a CD4 helper-type T cell producing IFN and IL-2 is protective, whereas in other experimental malaria models evidence has recently emerged that CD8 cytotoxic T cells may be critically involved in protection.

Studies on vector biology in endemic areas of onchocerciasis in Ecuador revealed that *Simulium exigeum* has high susceptibility to the parasite, whereas the susceptibility of *S. quadrivittatum* is quite low.

In terms of pathogenesis, filarids were found to metabolize arachidonate into pharmacologically active eicosanoid derivatives that could modify interactions of filariae with a wide variety of host cells. In terms of host-vector interactions and parasite-vector interactions, several models using *Dirofilaria*-susceptible or -resistant mosquitoes have established differences in hemocyte function related to tyrosine and dopa metabolism which may correlate with resistance. Finally, molecular differences in the transcription of RNA in thoracic musculature of susceptible versus resistant mosquitoes infected with *B. malayi* or malpighian tubules of those infected with *D. immitis* have been observed and are under study.

These examples indicate that major strides in integrating the most modern molecular genetic and immunological approaches to attacking these major pathogens of man have been made by scientists of both Panels over the past 5 years. In this spirit, it is perhaps noteworthy in the annual report from one of the Panels, that the usual organization by parasite species has been discarded, and the highlights presented across genus and species under the biologically significant categories of general biology, immunology, molecular biology, parasite physiology and drug development, pathogenesis, and immunoregulation.

2. The goal of the Joint Panels on Parasitic Diseases is to increase understanding of vector-borne helminthic and protozoal diseases, thereby allowing development of effective modes of prevention or treatment of these major worldwide threats to human health. The intricate interdependence and interactions of these parasites with their vectors and hosts present some of the most difficult and challenging problems of modern biology, requiring the application of the most sophisticated approaches of molecular biology, immunology, epidemiology, cell biology, pharmacology, and parasitology. While the problems of the individual parasites, vectors, and diseases are indeed unique, the approaches, to solving them cut across disciplinary and taxonomic lines. In the past 5 years, remarkable progress has been made in bringing the new biomedical technologies to bear on parasitic diseases and in providing better understanding of the immunology, molecular biology, epidemiology, and pathophysiology of schistosomiasis, filariasis, and malaria as a result of the work of these Panels.

3. At the time of the previous review, the importance of the scientific contributions of the Parasitic Diseases Panels was recognized, and the Program's contract support for supply of biological materials to facilitate research by laboratories not having access to various stages of parasite life cycles was acknowledged. However, at that time it was clear that newer technologies, particularly the use of monoclonal antibodies and recombinant DNA technologies were just being introduced into the study of protozoal infections, and that these new molecular and genetic tools and approaches were likely to have great relevance to the study of helminths as well. Because of the acknowledged expertise within both countries in malaria re-

search, the recognition of malaria infections as being a major cause of morbidity and mortality, and because new molecular tools and approaches likely to be relevant to helminths were just being applied to plasmodia and other protozoans, the guidelines of the panels were expanded to include research on malaria, and to a lesser extent other protozoal infections. A symposium on *Current and Unresolved Problems in Parasitic Diseases* was held by the Joint Parasitic Diseases Panels at the NIH in 1983 in which a wide variety of new molecular, epidemiological, and immunological approaches being implemented or contemplated over the next several years were presented to the Joint Panels. Following that meeting, the expanded guidelines for the Panels were drawn up. In the subsequent 5 years, remarkable advances in the application of modern immunology and molecular biology have been made in schistosomiasis and filariasis, as well as in hemoprotozoan diseases. These advances have been facilitated by efforts of the Joint Panels working under the new guidelines.

The revised guidelines and objectives strengthening the application of advanced molecular biological and immunological approaches not only to complex parasitic metazoan, but also protozoan infections have brought forth a plethora of new insights and observations, indicating that the Panels are on an appropriate course.

It was the hope at the time of the previous review that broadening the guidelines to include protozoal diseases and fostering expanded scientific symposia and presentations on a wider variety of experimental systems would facilitate the transfer of knowledge of successful techniques and approaches in any one area to scientists working on related problems in different parasite or vector systems. The remarkable progress in all areas over the past 5 years has fully justified this approach.

4. The Joint Parasitic Diseases Panels are bringing to bear very high levels of expertise, the most sophisticated new approaches, and renewed vigor to these very difficult and burdensome parasitic pathogens of people of Asia and of the developing world. The research is highly integrated and interactive and yet clearly well-focused scientifically. The Panels presentations and progress confirm the value of the integration of the helminths and some protozoa and confirm the interdisciplinary and scientific interactiveness of the scientists associated with these Panels. Without a doubt, continuation of the approaches now being utilized by these panels will facilitate achievement of the above described goals.

The definition of antigens recognized by antibodies and T-cells, immunologic interactions, and idiotypic networks are fundamental in developing new tools for immunodiagnosis and vaccines. The further characterization of many of these antigens in terms of their biological and enzymatic function offers unanticipated new opportunities for drug design. The organisms and scientific problems are highly complex, but their study is yielding important new insights to the modern approaches taken by the Panels.

5. While productivity has been strong in the past 5 years, the manpower in this area is relatively limited. It is gratifying that new young scientists are being involved in the activity of these Panels. It can be anticipated in the next 5 years that significant advances will be made in serological and molecular diagnostic techniques, possible vaccine candidates, new drug design, and mechanisms for resistance; yet, a great deal more understanding and work will be necessary before these can be directly applied to prevention and treatment of human disease. With the limited support available for tropical diseases research worldwide, there is an urgent need to maintain and strengthen the work of these Panels.

It is the overall judgment of these reviewers that the Panels on Parasitic Diseases are making an important contribution in the spirit of the U.S.-Japan Cooperative Medical Science Program, and that they should be commended and enthusiastically supported.

Leprosy Panels

Panel Reviewers: Dr. Tadao Shimao
Dr. Edward W. Hook

1. Leprosy remains a major world health problem involving millions of persons in many countries. During the years covered by this report, 1982–1987, extraordinary progress has been made by participants of the program in the acquisition of fundamental knowledge related to leprosy. These new basic observations, especially those that relate to the antigenic composition of *Mycobacterium leprae*, are directly relevant to the overall objective of eradication of this disease. Overall, the studies, which have in recent years emphasized the immunology and chemotherapy of leprosy, are directly and fundamentally linked to the Program's objectives to develop effective means to prevent and control leprosy. Investigations carried out during the past 5 years hold promise for the development of new vaccines and new chemotherapeutic agents.

During the period under review, 1982–1987, there have been unprecedented accomplishments in the broad area of leprosy research. The most significant accomplishments, reported by the U.S. participants, were in the areas of molecular biology of *M. leprae* and in the definition of its antigens by chemical means, the use of monoclonal antibodies, and T cell lines and clones. Very significant progress was also made in the areas of immunodiagnosis, immunopathology, and immunoregulation. Japanese participants in the program focused on the growth of the bacillus, study of its metabolism, development of various rodent models to *M. leprae*, and chemotherapy. Their work did not emphasize molecular biology or cellular immunology. A truly joint effort by U.S. and Japanese participants involved chemical synthesis of the sugar epitopes of the phenolic glycolipid and their application to serodiagnosis of lepromatous leprosy.

Presentations and statements of panel members and chairmen over the years make it clear that the antigenic analysis of *M. leprae* continues to be a top priority of the U.S. contingent. They are committed to the use of modern molecular biology, monoclonal antibodies, cloned T cells, and chemical tools in the further definition of the key antigen determinants, both peptide and carbohydrate, involved in cellular and humoral immunity and from this the development of *in vitro* and *in vivo* diagnostic tools and the ultimate provision of subunit vaccines.

2. The panels' scientific program has fulfilled many of the objectives listed in the guidelines, particularly the revised guidelines. During the past 5 years, there has been extraordinary progress in the identification of antigens of *M. leprae*, which in turn holds promise as the basis for development of new, more effective vaccines. In this respect, most of the significant developments have come from U.S. and European workers. With the exception of work on the use of streptomyces for the expression of the *M. leprae* genome, Japanese leprol-

ogists have not participated extensively in the burgeoning use of molecular biology in leprosy research. The reviewers recommend greater emphasis on the molecular approach and the use of T-cell clones and lines in efforts to define the determinants involved in cell-mediated immunity, whether it be protective or suppressive. Already among several key Japanese participants and also some U.S. workers, there perhaps has been overemphasis on the serodiagnosis of leprosy; serology in general applies only to the most identifiable, lepromatous, form of disease and is consequently only of limited usefulness.

There has been discussion in the past year of the possibility of combining the Tuberculosis and Leprosy Panels of the U.S.-Japan Cooperative Medical Science Program. This notion is based on the fact that these mycobacterial diseases share many common immunopathologic features and on the feeling that interaction of the scientists who comprise the mycobacterial panels could be mutually stimulatory and advantageous. Already, by convening adjoining meetings and holding joint sessions, the panels are achieving these worthy objectives. Actual amalgamation of the panels might in fact be detrimental in that large segments of Japanese and U.S. workers concerned with the diseases, pathology, and treatment, rather than the relevant bacilli, would feel excluded. Moreover, prelimimary discussions indicate that the suggestion to combine the panels will not receive widespread support and will endanger the concept of sharing. As indicated above, there already is a good degree of complementarity in the work fostered by the two panels, and there is genuine cooperation in some areas (e.g., the whole phenolic glycolipid story). Obviously, there is a need for greater cooperation and a change in the focus of the Japanese Leprosy Panel. The present format is more suited to accommodating these adjustments. However, it is recommended that the Leprosy and Tuberculosis Panels continue to hold adjoining meetings and joint sessions.

3. The objectives listed in the revised Leprosy Panel Guidelines are appropriate. The technology is available to achieve the objectives. However, many laboratories and major programs are not availing themselves of this new technology. Throughout the report, recommendations have been made concerning the reorientation of these programs.

4. The approaches employed during the past 5 years, in conjunction with the recommendations made in this report, should ensure progress towards the achievement of the panels' objectives. Future work will consist of continued evaluation of the immune responses to the antigens of *M. leprae*, further definition of immunopathological mechanisms, and the development of new vaccines and chemotherapeutic combinations.

5. It is unlikely that the objectives will be reached within the next 5 years. The timeframe for when the panels' objectives can or should be modified or terminated should be assessed at the annual meeting of the combined panels.

It is the opinion of the reviewers that the work of the current Leprosy Panels is extremely important, since it promises to provide measures to alleviate a major health problem affecting large portions of the world's population. Evidence supports the position that progress in the past 5 years has exceeded that recorded for any other 5-year period in the panels' history; as indicated above, some key developments have been of great potential importance. Nevertheless, there should be certain changes in direction and a reorganization of priorities. The reviewers recommend approval of the revised guidelines. Full support for this program is recommended for an indefinite period of time.

Tuberculosis Panels

Panel Reviewers: Dr. Hideo Fukumi
Dr. Robert E. Shope

1. Twenty-three years ago when the United States and Japan undertook the Cooperative Medical Science Program, not only to alleviate disease in the two host countries, but also in Asia, tuberculosis was recognized as a major problem. It continues to be a significant and, indeed, an increasing problem. The failure of the BCG trial in south India to demonstrate efficacy has highlighted the need for improved immunoprophylaxis. The continued rapid increase in numbers of AIDS cases in the United States and other parts of the world has produced a growing population at risk of reactivation of existing tuberculosis or susceptible to disease from infection with *M. tuberculosis* and *M. avium* complex organisms. The research areas outlined in the Panels' guidelines are more than ever relevant and appropriate for the U.S.-Japan Cooperative Medical Science Program.

2. The Panels have made outstanding progress in the areas of identification of biologically and immunologically active constituents of the tubercle bacillus and related organisms. They have vigorously made use of the modern biotechnology, including monoclonal antibodies and recombinant DNA, to approach this area. The Panels have also made effective progress in the field of immunology of tuberculosis. Of note are the discovery of a new lymphokine and the development and exploitation of PPD-reactive T-cell clones to investigate immunological phenomena. The Panels have also addressed each of the other areas of the guidelines either directly or indirectly. The research has been effective, although in some cases limited. Limitation in the numbers of available investigators has prevented more rapid progress in areas such as vaccines and adjuvants. These, however, are of high priority and should not be eliminated from the guidelines.

3. The objectives as stated in the guidelines revised in 1978 are still appropriate. The previous review pointed out the need to use monoclonal antibodies to identify specific moieties to differentiate *Mycobacterium* species and to select for these moieties by affinity chromatography. It also urged application of recombinant DNA techniques to obtain abundant supplies of antigens. Good progress in these directions is evident, and the approaches continue to be timely. The progress in identifying antigens has been gratifying. It is opportune now to apply this new molecular knowledge of the constituents of the tubercle bacillus and related organisms to studies of immunoprophylaxis. It should be noted that studies of adjuvant properties of mycobacteria, if leading to improved adjuvants for molecular vaccines (including vaccines for AIDS), would provide a critically needed breakthrough, a much wider application not limited to the control of tuberculosis. The study of the molecular genetics of *Mycobacterium* is in its infancy and should be emphasized during the coming 5 year period.

4. The biotechnical approach including modern immunological methods, monoclonal antibodies, recombinant DNA,

nucleic acid/carbohydrate/protein synthesis, and advanced enzymology will continue to lead to progress. Animal models are essential.

5. Tuberculosis and related diseases continue to increase and attempts to control them should continue as long as the diseases are prevalent. The most important goals have not changed in 5 years—immunization is still paramount, even more important than improved chemotherapy. Both goals are important, however, and neither is likely to be perfected in the next 5 years. Other goals such as specific diagnostic reagents, perhaps achieved through synthesis of specific epitopes, should be reached prior to the time of the next review. The Panels serves as a focus for many other researchers, some from other nations, and some in related but different fields. These make up a critical mass needed to solve the complicated problems posed by *Mycobacterium* species. The Tuberculosis Panels are an important and necessary part of the overall solution.

Since the last report on the review of activities of the Tuberculosis Panels, the tools of recombinant DNA and hybridoma biotechnologies have been increasingly applied to the study of *Mycobacterium tuberculosis*, opening important new approaches. At the same time, the appearance of AIDS and the deterioration of public health efforts in some troubled parts of the world have led to resurgence of tuberculosis and related mycobacterial disease. Thus, the paradox that in spite of the application of the most modern technology to the study of the pathogenesis, immunology, and epidemiology of mycobacterial disease, the net result has been an increase in morbidity and mortality. The increase has been dramatic in Africa, significant in the United States, and a threat in Japan which still registered in excess of 400,000 tuberculosis patients in the mid-1980's. The meetings of the Joint Panels have alternated between Japan and the United States. Attendance has been excellent, approaching 100 each year, mostly from the host country, but with representative experts from both nations. The 1987 meeting was held jointly with the Leprosy Panels which share many similar problems and methodologic approaches with the Tuberculosis Panels.

ACQUIRED IMMUNODEFICIENCY SYNDROME PANELS

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Profiles

U.S. AIDS PANEL

Ashley T. Haase
Chairman (1988–Present)

Ashley T. Haase, Professor and Head of the Microbiology Department at the University of Minnesota, received his M.D. from Columbia University College of Physicians and Surgeons, completed an internship and residency at the Johns Hopkins Medical Institutions, and subsequently trained in virology with H.G. Pereira at the National Institute for Medical Research in London and Sam Baron at the National Institutes of Health. He came to to Minnesota from the University of California, San Francisco, where he was professor of medicine and microbiology, as well as chief of infectious diseases at the Veterans Administration Hospital. Doctor Haase is a memeber of the National Advisory Council of the National Institute of Allergy and Infectious Diseases (NIAID), and both a Javits Neuroscience Investigator of the National Institute of Neurological and Communicative Disorders and Stroke and a MERIT awardee of NIAID. He is chairman of the AIDS Panel of the U.S.-Japan Cooperative Medical Science Program.

JAPANESE AIDS PANEL

Dr. Yuichi Shiokawa
Chairman (1987–Present)

Dr. Yuichi Shiokawa graduated from the Medical Faculty, University of Tokyo, in 1941. His studies included infectious diseases, collagen diseases, and allergology in Second Department of Medicine, University of Tokyo, and he has been instrumental in the development and progress of these areas in Japan, especially in the control of rheumatic fever. Dr. Shiokawa established the concept of malignant rheumatoid arthritis as a new disease entity, resulting in the development of control measures, for which he was awarded the Japan Rheumatism Association Prize. He was appointed to a professorship in the Department of Allergological Medicine in Juntendo University Medical School. He served as the member of Public Health and Pharmaceutical Councils, Ministry of Health and Welfare (MHW), Government of Japan. Since 1984, Dr. Shiokawa has chaired the MHW AIDS Working Group and, after its reorganization in 1987, held the chairmanships of GOJ AIDS Expert Committee, MHW AIDS Control Committee, and MHW AIDS Surveillance Committee. He is a leader in all aspects of AIDS studies and was responsible for the establishment of the present system of AIDS prevention and control in Japan.

Dr. Shiokawa holds the titles of Honorary President of Japan Rheumatism Society, Chairman of the Japan Rheumatism Foundation, Chairman of the Japanese Society of Inflammatology, and Honorary Member of American Rheumatism Society, and is still serving as an active and leading figure in Japan in a broad area including clinical immunology, allergology, and inflammatology as well as in AIDS. A number of standard textbooks covering collagen diseases and rheumatism have been published by Dr. Shiokawa. The Japanese people are proud to have him as the first chairman of the AIDS Panel.

GUIDELINES

ACQUIRED IMMUNE DEFICIENCY SYNDROME PANELS
United States-Japan Cooperative Medical Science Program

Acquired Immune Deficiency Syndrome (AIDS) is a newly recognized disease that destroys the body's capacity to overcome a variety of infections. AIDS has become a global pandemic, with cases reported in more than 104 countries representing all continents. International studies have yielded important information on the natural history, epidemiology, clinical immunology, and etiology of AIDS. Studies in Southeast Asia, Africa, the Caribbean, and the United States have provided insights into the modes of disease transmissions; the possible role of parasitic, viral, and nutritional factors; the possible role of racial or genetic factors influencing exposure, infection, and disease expression; and cultural practices. A collaborative effort by Japanese and U.S. biomedical scientists would be of enormous value in solving the multiple problems relevant to the effective prevention and treatment of AIDS. The rapidity of the increase in diagnosed cases of AIDS and the morbidity of this devastating *disease* require the mobilization of the most creative scientists. Their objectives will be to aggressively pursue an intensive program aimed at developing new strategies and uncovering the clues necessary for providing effective treatment and prevention of this epidemic scourge. Research areas of high interest to the Panels are:

1. Development of antiviral compounds, vaccines, and immunomodulators for primary prevention and treatment, especially for secondary intervention of disease manifestation from virus carriers. Establishment of *in vitro* and *in vivo* evaluation systems, including the development of animal models.
2. To study the pathogenesis of AIDS: cell biology, virology, genetics, molecular biology, and immunological characterizations of HIV and related retroviruses aimed at improving the basic understanding of the causative agents.
3. To conduct clinical surveillance and epidemiological studies, including modes of transmission in Japan, the United States, and Asian countries in order to identify risk factors of infections caused by the human immunodeficiency virus (HIV) and other retroviruses, including further development of diagnostic test reagents.
4. To perform follow-up studies designed to determine the incidence of immunodeficiency and neurological disorders as a sequela of chronic HIV infections.
5. To identify limited, but sufficiently large cohort groups with high probability of developing HIV infection which can be followed prospectively and longitudinally for an indefinite but sufficiently long timeframe.
6. To exchange basic and clinical information between investigators of Japan and the United States and to share techniques and materials pertinent to human retrovirus infections.

At the first Joint Panel Meeting in December 1988, in Tokyo, two other areas of research interest were recognized and were proposed to be added to the guidelines:

7. Elucidation of the ecology, epidemiology, pathology, virology, and prevention of diseases caused by other members of human retro-viruses, e.g., adult T-cell leukemia (ATL) and HTLV-1-associated myelopathy (HAM) by HTLV-1, principally by the Japanese Panel.
8. Elucidation of the rate, diagnosis, impacts, and control of mother-to-child transmission of HIV, principally by the U.S. Panel.

U.S. AIDS Panel

BROAD GOALS

The overall objectives of the Panel are to join in the common efforts of scientists worldwide to develop strategies to prevent and treat AIDS. The Panel believes that this can be achieved by discussion and collaboration between scientists focused on eight areas of particular relevance and interest. These eight areas are designated in the guidelines above.

PROGRESS IN PRIORITY AREAS

On December 5–7, 1988, the Japanese panel of the U.S.-Japan Cooperative Medical Science Program on AIDS hosted the first scientific meeting of the Panels at the National Cancer Center in Tokyo. Investigators from both countries presented overviews and detailed reports in sessions on the epidemiology of AIDS; current understanding of the causative agent, the human immunodeficiency virus (HIV); the pathogenesis of lentivirus infections; and prospects for interventions and control. Those reports, which constitute the major achievements of the Panels since their formation are summarized here.

Some 5–10 million individuals worldwide are infected, and by 1992 the toll in the United States alone is expected to be 365,000 cumulative cases and 263,000 deaths. More than 80,000 of these will occur in that year alone, making AIDS the leading cause of death and major contributor to health care costs ($5–$13 billion). However, it is in Africa and other developing regions of the world where AIDS is expected to have its greatest impact. The reservoir of infected but asymptomatic individuals is probably in the range of 3–5 million with more than 100,000 cases of AIDS in central Africa to date. Further studies are needed of a related retrovirus, HIV-2, identified in West Africa in 1986 to assess its probable contribution to the AIDS epidemic.

HIV is transmitted sexually, parenterally, and perinatally. In the United States, the majority of HIV seropositive individuals are homosexual or bisexual males, but recent behavior modification has led to a decrease from 70% to 62% of the infected population. The incidence amongst IV drugusers and their sexual partners, on the other hand, is increasing (now about 30%) with a disproportionate share in Black and Hispanic residents of the inner city. With a 30%–50% transmission rate perinatally the number of pediatric AIDS cases is expected to rise dramatically in this group.

In Africa, the male to female ratio of infection, in contrast to the United States, is close to one, reflecting the high incidence of sexually transmitted diseases, the high incidence of infection in female prostitutes, and greater likelihood for transmission with associated genital ulcerations. The high incidence of heterosexual transmission from this large reservoir, and the 5%–10% seropositive blood donors, are the source of the somber predictions for the impact of AIDS in Africa.

Hopefully, the dissemination of information now underway in the United States, described by Dr. Allen, and outreach programs like the one at UCLA described by Dr. Fahey, with appropriate behavior modification can constrain the dissemination of HIV elsewhere.

The genetic organization and gene products of lentiviruses have, in common with other retroviruses, a 5' LTR with cis regulatory sequences; a pol gene encoding a polymerase with reverse transcriptase, RNase H, and endonuclease activities required for synthesis and integration of viral DNA, and genes encoding the structural polypeptides that comprise the nucleocapsid (gag) and envelope (env). The env product is also involved in cell fusion and killing, the biological activities in tissue culture characteristic of lentiviruses. There are additional open reading frames in HIV which encode products required for infectivity (vif) and regulation of gene expression. The tat and rev gene products regulate the abundancy and translational efficiency of spliced and unspliced irion mRNAs; the nef gene product acts to down regulate gene expression at the level of initiation of transcription. While the detailed molecular mechanisms of action of these lentivirus-specific genes have not as yet been completely defined, they are likely to be intriguing and novel.

Lentiviruses kill cells in culture and cause slowly evolving diseases affecting primarily the pulmonary immune and central nervous systems of man, primates, cats, and ruminants. Haase's review on the distinctive aspects of the pathogenesis of animal lentivirus infections underscores the great difficulties in preventing transmission and disease. Lentiviruses such as visna in sheep establish persistent infections in this host through down-regulation of viral gene expression which renders the infected cell a poor target for detection and destruction by immune surveillance. In mobile cells, particularly monocytes, infection is transmitted in and between individuals by a Trojan Horse mechanism in which the host humoral and cellular immune defenses fail to detect and eliminate the silently infected cells. Infections are not truly latent, however, as some infected cells produce viral gene products and elicit an immunopathological response which, in time, causes sufficient tissue damage to be manifested chemically. In visna and the progressive pneumonias of sheep, the inflammatory lesions in lungs and central nervous system (CNS) eventually lead to shortness of breath and paralysis. In infections by the Simian and feline lentiviruses, involvement of the immune system is as prominent as it is in HIV infections. The striking parallels to HIV infection support the use of these animal models in studies of pathogenesis of lentivirus infections with particular relevance to AIDS.

During the course of HIV infection there is both a humoral and cellular immune response. Neutralizing antibody directed to gp120, the external portion of the env gene product, virus-specific cytotoxic lymphocytes and antibody-dependent cell-mediated cytotoxicity have been documented, but, as is true in the animal models, virus is not eradicated. Eventually, with an incubation period of 5–10 years, CD4+ T helper cells (T4) are depleted and opportunistic infections appear where the T4 count is 200/mm3. The immunological response to infection and associated alterations in immune function were reviewed and the utility of following the T4/T8 ratio, T4 count, and various independent measures of immunopathological events in lymphocyte turnover (increase in b2-microglobulin and neopterin) and activation of myeloid cells (release of an IL-2 receptor characterized, and described by Honda et al.) in diagnosis, prognosis, treatment, epidemiology, and research was stressed. Although the mechanisms involved in these virally induced alterations in the nervous system are still poorly understood, the changes in these parameters are strongly correlated with the development of disease and response to treatment.

Thus far, lymphokines and colony-stimulating factors have shown activity in tissue culture but have proved disappointing *in vivo*. Infusion of CD8+ lymphocytes was also discussed since they suppress HIV replication *in vitro* and HIV and SIV (Kannagi, Harada, and Letvin) are more readily isolated in CD8-depleted individuals.

Vaccine development is now largely centered on recombinant gp120 and identification of epitopes involved in viral neutralization. Sequence homology analysis suggests that a cellular protease may recognize a portion of gp120 and activate fusion and entry of virus by cleavage. Inhibition of giant cell formation by a competitor peptide designed to examine this region of gp120 illustrate the potential of this kind of approach to the design of new drugs.

Recombinant CD4 may also block attachment of HIV to T4 cells, but once virus has entered the cell, effective interventions must be directed at selective inhibition of other stages of viral replication. Richman provided a progress report on the treatment of HIV infections, particularly focused on AZT and other inhibitors of viral DNA synthesis such as foscarnet, dideoxycytosine, and dideoxyinosine. Alternatives will clearly be needed in light of Richman's recent finding of strains of HIV resistant to AZT. Still later stages in the viral lifecycle may be inhibited by interferons and inhibitors of glycosylation. The latter interfere with assembly or processing of gp120. It was evident from the presentations of Yamamoto et al., on tissue culture screening systems, Yamada on clinical studies in HIV-infected hemophiliacs, and W. Parks on AIDS clinical trials groups, that in both Japan and the United States there is both commitment and appropriate structures to continue the search for effective measures against HIV.

PROJECTS SUPPORTED BY THE PROGRAM

GRANTS

Investigator and Institution	*Title*
John L. Fahey University of California Los Angeles	Clinical and Fundamental Immunology
	Interdisciplinary Research of Immunologic Diseases
	Center for Interdisciplinary Research in Immunologic Diseases
	Studies of Acquired Immunodeficiency Syndrome
Ashley T. Hasse University of Minnesota	Pathogenesis of the AIDS Dementia Complex
	Pathogenesis of HIV Induced Immunodeficiency
Beatrice H. Hahn University of Alabama Birmingham	Studies of HTLV-III/LAV Envelope Variation
	Molecular Structure-Function Studies of HTLV-4 & HIV
	HIV Replication and Pathogenesis
	Birmingham NCDDG
	Mucosal and Systematic Immunity to SIV and HIV Vaccines
Wade Parks University of Miami	Acquired Immunodeficiency Disease in Haitian Children
	Infants of HTLV-III/LAV Seropositive Mothers
	Use of Analogs of Myristic Acid to Modulate HIV-1 Assembly/Replication
Lee Ratner Washington University	Pathogenesis of Human Immunodeficiency Virus Infection
	AIDS Clinical Study Group
	Use of Analogs of Myristic Acid to Modulate HIV-1 Assembly/Replication
	Inhibitors of Retroviral Protease

Douglas Richman VA Medical Center San Diego	Mechanisms of Resistance in Clinical Isolates of HIV

CONTRACTS

Thomas C. Quinn NIAID Intramural Research Program Project Officer	Interagency Agreement: Epidemiologic, Virologic, and Immunologic Aspects of HIV Infection in Zaire Maryland Medical Laboratories: *In vitro* Cultivation and Viral Characterization of HIV Isolates Johns Hopkins University: The Immunologic Response of Herpesvirus Infections and Human Retroviral Infections (HIV/HTLV)
Douglas Richman VA Medical Center San Diego	Techniques to Rapid Viral Diagnosis Development of Screening Test for HTLV-III

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Japanese AIDS Panel

BROAD GOALS

When AIDS emerged as a new medical entity in 1981 and its pandemic expansion was recognized to be a new, global threat in 1984, cooperative activities in AIDS research between Japan and the United States were proposed and the possibility of establishing new panels on AIDS in the Japan-U.S. Cooperative Medical Science Program (CMSP) was discussed. In June 1987, on the occasion of the Third International Conference on AIDS in Washington, D.C., a preliminary meeting was held between groups of AIDS experts of Japan and the United States and an agreement was reached to establish AIDS Panels with the guidelines cited below.

The Subcommittee on Program Review and Planning of CMSP met in July 1987, in Bethesda, Maryland, and reached a consensus that AIDS Panels be added to the Program. Later, the Joint Delegation of the Program adopted this recommendation to establish new AIDS Panels.

Broad goals establishing research areas of high interest were incorporated into guidelines for both Panels.

In January 1988, five panel members were designated and the first meeting of Japanese Panel was held on January 27, 1988. The panel selected Dr. Yuichi Shiokawa (Juntendo University Medical School) as Chairman, Dr. Takashi Kitamura (National Institute of Health) as Secretary and 20 other scientific members. In April 1988, an AIDS Research Center consisting of 5 laboratories and 20 scientific staff was established in the National Institute of Health (JNIH), Tokyo, and was designated as the secretariat of the Japanese AIDS Panel.

PROGRESS IN PRIORITY AREAS

The Japanese AIDS Panel worked in cooperation with the AIDS Surveillance Committee of the Ministry of Health and Welfare (MHW), several working groups in various aspects of AIDS-sponsored by research grants from MHW, and the Japan AIDS Research Society (JARS) organized in December 1987. The first Joint Conference on AIDS and Joint AIDS Panel Meeting were held in Tokyo in December 1988, and the second Conference in January 1990, in New Orleans. Progress by the Japanese AIDS Panel reported or scheduled for those meetings is as follows:

1. Anti-HIV Chemotherapeutics

A. Screening System of Anti HIV Drugs:

Prof. N. Yamamoto's group (Yamaguchi University) developed a quantitative and reproducible system *in vitro* of screening anti-HIV chemotherapeutics employing a) HIV growth suppression in MT-4 cell line (human T-cell line transformed by HTLV-1 virus, having high sensitivity to HIV infection) and b) suppression of cell fusion by HIV between MT-4 cell lines of persistently infected and normal cells. Using their system, they screened a series of chemicals: A nucleoside analogue, 2,3-didehydro, 2,3-dideoxythimidine (DHT) and a sulfated polysacchaide, lentinan sulfate (LS), was found to be potent HIV replication inhibitors comparable with AZT. LS inhibited HIV-induced T-cell fusion, while DHT and AZT did not.

B. HIV Inhibitors through gp4l Glycosylation:

Group of JNIH (H.Shimizu et al.) screened a series of deoxynojirimycin derivatives in Yamamoto's system and found two derivatives to be potent HIV growth inhibitors. The mechanism of activity of one derivative (ND9OO) was analyzed and inhibition of glycosylation of an envelope protein, gp4l, was suggested to be the main cause of inhibition. Two glycosylation inhibitors allowed the formation and release of morphologically intact HIV virions in the first cycle of infection but formed virions were noninfectious and could not repeat the cycle.

C. Enhancement of HIV Replication by Tumor Necrosis Factor (TNF):

The group of N.Kobayashi et al. (Yamaguchi University) demonstrated that the addition of a monokine, TNF, enhanced the expression of HIV. HIV infection of MT-4 cells and resulting cytopathic effects were accelerated in the presence of TNF. The effect of TNF was observed within 1 hour after addition. The detailed mechanism of the enhancing effect of TNF on HIV infection remains to be elucidated, but their findings per se may suggest that some lymphokine(s) may serve in facilitating the progress of HIV infection.

2. Therapy of HIV Carriers and AIDS Patients

A. Treatment of AIDS Patients by CSF:

Dr. F.Takaku and colleagues (Tokyo University Medical School) tried recombinant glanulocytes colony stimulating factors (rCSFs), G-CSF, for the treatment of four AIDS cases. G-CSF significantly improved the number andh)function of granulocytes in clinical AIDS patients, and clinical conditions also improved. G-CSF may be useful in treating opportunistic infections in AIDS patients. Combined use with AZT may also be proposed, in both reducing the hypogranulocytosis as an adverse effect of AZT and potentiating the recovery from the opportunistic infections.

B. Cohort Study of Treating HIV-Infected Hemophiliacs:

In Japan, more than 90% of HIV carriers are found among hemophiliacs who were exposed to blood or blood products imported before 1984. MHW sponsors the research group led by Prof. K. Yamada (St. Marianna Medical School) aimed at introducing experimental therapies to prevent the development of clinical AIDS among hemophiliac HIV carriers. The group revealed that 1,600 (38.8%) of 4,000 hemophiliacs in Japan were infected with HIV before 1984, 51 AIDS patients (37 with hemophilia A and 14 with hemophilia B) have been identified among them and the following two measures to control the development of AIDS were introduced under the sponsorship of MHW; a) observation of the natural history of hemophiliac HIV carriers, by nationwide registration of cases and regular check of prognostic hematological, immunological, and virological parameters, b) installation of therapeutic and preventive measures, e.g. AZT, interferons, lymphokines, etc., suggested by basic and clinical research activities, and scientific evaluation of those interventions.

3. Vaccine Development

Monoclonal antibodies against HIV envelope glycoprotein have been studied extensively by the group of Dr. T.Hattori (Kumamoto University Medical School) in special reference to their potency of interfering with HIV growth. Their activities were analyzed in parallel by several other groups in relation to possible activities on the regulatory proteins. Demonstration of the efficacy of HIV glycoproteins produced by recombinant vaccinia or baculoviruses with incorporated HIV env gene(s) were tried by a number of groups. Groups at Kyoto University and JNIH could induce antibodies in animals to react with the original strain of HIV.

4. Virology of HIV and Related Viruses

A. Heterogeneity of Biological Properties of HIV Isolates:

Prof. T. Kurimura and his colleagues (Tottori University Medical School) compared the growth capabilities in a T cell line (MT-4) and peripheral blood mononuclear cells (PBMC) among HIV isolates from cases of various stages of HIV infection. Viruses isolated from symptomatic carriers grow well in both PBMC and MT-4 cells, while the majority of viruses isolated from asymptomatic carriers grew well only in PBMC. The change of HIV population in such a growth characteristic was observed in each of the individual carriers during the shift from asymptomatic to symptomatic status and prognostic significance of the population check through this marker was suggested. Analysis of the change in gene structure associated with such a shift is in progress in JNIH. Dr. H.Tsuchie (JNIH) traveled to the United States in March 1989, in an effort to collect HIV isolates from cases of various risk groups and of various stages in cooperation with U.S. panel members. Dr. S. Harada (Kyoto University) observed also the heterogeneity of biological characteristics among plaque-cloned HIV populations. Clones could be grouped into large plaque-producing and small plaque-producing ones, the former could replicate more rapidly than the latter.

B. Regulation of HIV Gene Expression:

Dr. T. Okamoto's group (National Cancer Center, Tokyo) analyzed intranuclear transactivation of HIV-gene expression in an *in vitro* system, and the role of enhancer sequence within HIV-1 LTR was confirmed. Lymphotoxin, one of cytokines produced by HIV-carrying cells was suggested to play an important role in cytotoxic effect of HIV. Dr. M. Hatanaka's group in Kyoto University analyzed the functions of transactivating regulatory gene products, tat-IV and art/trs and demonstrated that the basic residue-rich region of these proteins has the function of a nucleolar targeting signal and site-directed mutagenesis of the region correlated directly with the transactivating function. The detailed mechanism of the shift from latency to lytic growth was proposed on the basis of these studies.

C. Genetic Analysis of SIV AGM and SIV MND:

The group of Dr. M. Hayami (Tokyo University Institute of Medical Sciences) isolated 20.SIV AGM from naturally infected asymptomatic AGM and two SIV.MND from wild caught mandrills. Genomic structures of SIV.AGM isolates were similar to other members of HIV/SIV but lacked an R region and was almost equally distant from HIV-1, HIV-2, and SIV.MAC, which does not support the prevalent concepts or theories that SIV and HIV-2 form one group. SIV.MND was identified to be a new member of HIV/SIV group, equally distant from other HIV/SIV members including SIV.AGM. These results are in clear contrast to the prevailing hypotheses that HIV was originated from SIV.

5. Animal Models:

A. HIV/SIV in Non-human Primates:

The group of Dr. M. Hayami (Tokyo University Institute of Medical Sciences) in cooperation with people of JNIH tested experimental inoculation of SIV.AGM and SIV.MND in several species of monkeys. SIV.AGM could infect the AGM and cynomolgus monkey, and SIV.MND infected the cotton-top tamarin, but no clinical signs were observed in these monkeys. Inoculation of HIV-2 into the cynomolgus monkey could induce low levels of antibody response, but no clinical signs nor physiological changes were observed.

B. Feline Immunodeficiency Virus (FIV) Infection of Cats:

Dr. T. Ishida (Nippon Veterinary and Zootechnical College, Tokyo) has demonstrated a high prevalence of FIV infection among Japanese domestic cats. Experimental infection of SPF cats with FIV induced the development of the initial acute phase of FIV disease, with a 40–50 week duration, characterized by lymphadenopathy, cyclic neutrophilia, fever, and chronic diarrhea, followed by the asymptomatic carrier phase. More severe illness, characterized by the presence of anemia, emaciation, opportunistic infections, and/or malignancies was seen in 7% of the 700 infected cats. These cats exhibited identical symptoms with human AIDS cases, and 75% of such cases died within 6 months. FIV infection caused a clinical syndrome similar to human AIDS caused by HIV and its possibility of being an animal model of human AIDS was strongly suggested.

C. Mouse AIDS (MAIDS) as an Animal Model of Testing Possible AIDS Drugs:

The LP-BM5 murine leukemia virus complex can induce immunodeficiency reminiscent of human AIDS in C57BL/10 mice, i.e., abnormal lymphoproliferation and severe, immunodeficiency in an early stage. The group of JNIH (Drs. Ohnoda, Mizuochi, et al.) used this system for the *in vivo* screening of anti-HIV drugs. Simultaneous or prior peroral administration of AZT to LP-BM5 virus inoculation could prevent the development of immunodeficiency, but a later start of AZT administration reduced the therapeutic effects significantly; starting 1 week later, only a partial prevention was noticed, and by starting 2 weeks later, almost no therapeutic effect was observed. The usefulness of the MAIDS system as an *in vivo* step of anti-HIV drug screening was strongly suggested.

6. Diagnostic Methods and Reagents:

A. Gelatin Particle Agglutination (GPA) Test:

A test utilizing agglutination of HIV protein-coated gelatin particles was developed for the detection of anti-HIV antibodies in cooperation with Dr. Kobayashi (Yamaguchi University) and Fuji Revio, Co., Tokyo, as an analogy of the method developed against HTLV-1. GPA has similar sensitivity and specificity comparable with conventional EIA procedures used in the diagnostic tests and requires no incubator or reader, and is regarded as a suitable method to be used in field conditions in Southeast Asia and Africa. The Japanese Government is supporting the promotion of GPA through contributions to WHO's Global Programme on AIDS and ODA's.

B. Polymerase Chain Reaction (PCR) for the Detection of HIV Prior to Seroconversion:

Detection of the HIV genome in plasma or tissues of infected individuals is recognized as a reliable and sensitive method of diagnosis prior to seroconversion, and numerous studies have been reported after the original development of PCR by Ou in 1988. In Japan, numerous laboratories including those in JNIH, Tottori University, and Kumamoto University have reported their results during the Third Congress Meeting of JARS, July 1989, Matsue, and several points to be solved before its being employed extensively were discussed. Improvement of PCR by the use of a nonradioactive probe and a heightened level of detection was reported by the group of JNIH (Dr. K. Yamaguchi, et al.) in the Congress Meeting of Japan STC Society, December 1989, Tokyo.

C. Sensitive Procedures of Antigen Detection:

A group of JNIH (Dr. H. Mizutani et al.) reported a high sensitivity detection of HIV antigen by laser-magnetic immunoassay (LMI), where 0.01 picogram/ml of HIV virion protein could be detected. LMI employs magnetic-labeled antibody against HIV which later can be quantitated by excitation by laser beam. The LMI equipment may be improved in its size and cost if used by large numbers of workers, and the usefulness of LMI in antigen screening of donated blood samples is suggested.

7. Epidemiology:

A. Estimation of Trends of AIDS and HIV Infection in Japan:

A working group led by Prof. K. Soda (Yokohama City University School of Medicine) reported a cooperative study estimating a future trend in HIV infection in Japan: a) There are 5,000 hemophiliacs in Japan, 40% of which are estimated to be HIV-infected. b) Assuming that the population of male homosexuals is 300,000 and 10% of which are promiscuous, the cumulative number of seropositives at the end of 1987 and 1992 are estimated to be 370 and 910, respectively. c) Estimation of HIV prevalence among other risk groups (IVDU, prison inmates, and prostitutes) was difficult due to lack of epidemiological information, but fragmentary study results are suggesting extremely low levels of prevalence. d) Heterosexual transmission to Japanese citizens outside Japan is slow, but has been steadily rising since 1988. e) The total number of HIV seropositives is estimated to be about 3,000 by the end of 1992, and the cumulative number of AIDS patients to be between 260 and 300. Further studies in updating the approach and information collection are necessary.

B. Epidemiology in Southeast Asia:

Prof. K. Soda (Yokohama University Medical School) visited Southeast Asia in March 1989, for a CMSP-sponsored epidemiological study. He has done studies in WHO's Western Pacific Regional Office, Manila, Philippines, and health ministries, national institutes, and universities in Philippines, Singapore, and Thailand. He collected epidemiological information indicating that Thailand entered the AIDS epidemic after July 1988, and effective domestic and international control measures are urgently needed. All Southeastern Asian countries may not be able to escape the fate seen in Thailand unless an internationally coordinated control program is installed.

8. HTLV-1 Infections:

Adult T-cell leukemia (ATL) has been recognized to be endemic in southwestern Japan, and its ecology and clinical identity were established by clinical groups in Kumamoto University (Dr. K. Takatsuki) and Kochi University (Dr. K. Miyoshi). Its etiology involving a transmissible agent(s) was established by the groups in Kumamoto, Kochi, and Kyushu Universities (Dr. K. Okochi), and the agent was identified to be HTLV-1 by Prof. Y. Hinuma (Kyoto University Institute of Virology). Dr. M. Osame (Kagoshima University Medical School) established the HTLV-1 etiology of spastic myelopathy endemic in the southwestern Japan and named its as HTLV-associated myelopathy (HAM). HAM was studied in cooperation with groups in the United States which had recognized another spastic myelopathic disease, tropical spastic paraparesis (TSP) in Carribean and West African countries, and the similarity of TSP with HAM was established.

Groups in Nagasaki, Kumamoto, and Kagoshima have demonstrated that avoidance of breastfeeding by HTLV-carrying mothers is the best measure of preventing vertical transmission of HTLV-1 and that ATL & HAM can be eliminated from the blood supply by effective screening of HTLV carriers. In the First and Second Joint Meetings of AIDS Panels, Prof. Takatsuki (Kumamoto University) reported the compiled achievements in ecology, epidemiology, pathology, and clinical hematology of ATL and Professors Y. Iwasaki (Tohoku University Medical School) and M. Osame reported the pathology, immunology, and virology of HAM.

SIGNIFICANT ACCOMPLISHMENTS

Genetic Relation of SIV from African Green Monkey and that from Mandrill with other HIV/SIV

AIDS was reported in 1980 in the United States, and its causative agent, HIV was isolated in 1983. Soon after HIV was isolated, workers demonstrated that several species of nonhuman primates were naturally infected with HIV-related viruses, now called SIV. It was suspected that HIV was transmitted from monkeys to humans explaining its sudden appearance in man. The origin of HIV was first hypothesized to be the African green monkey because 20%–30% of African green monkeys are naturally infected and do not manifest disease. We isolated and genomically analyzed SIV$_{\text{AGM}}$ from the African green monkey and SIV$_{\text{MND}}$ from wild mandrill of Africa.

Twenty isolates were obtained from animals originating in Kenya and Ethiopia. Five were molecularly cloned and one was totally sequenced. Genomic construction of SIV$_{\text{AGM}}$ is similar to HIV-1, HIV-2, and SIV$_{\text{MAC}}$, the first SIV isolate from a rhesus monkey kept in the colony of the New England Primate Center. There is, however, some difference in the open reading frame. The *pol* gene is rather conserved and generally indicative of a relationship among retroviruses. SIV$_{\text{MAC}}$ has very much higher homology to HIV-2, 80% in *pol* regions. SIV$_{\text{AGM}}$ has about 55% homology in *pol* regions to each virus. Thus, SIV$_{\text{AGM}}$ is equally distant to HIV-1, HIV-2, and SIV$_{\text{MAC}}$.

The consensus maps of cloned 5 SIV$_{\text{AGM}}$ isolates of different origins were constructed. SIV$_{\text{AGM}}$ has a great restriction site heterogeneity not only in the *env* region but in overall regions of the genome. These 5 SIV$_{\text{AGM}}$ isolates, however, belong to one virus group, because all hybridized strongly with a prototype, SIV$_{\text{AGM}}$ [TYO-1], and do not cross-react with other HIV/SIV DNA probes. They were more diversified than in the HIV-1 group and the HIV-2 group.

Following the SIV$_{\text{AGM}}$ isolation, we isolated 2 mandrill viruses and one of them SIV$_{\text{MND}}$ (GB-1) strain was totally sequenced. The genomic structure is almost the same as in other HIV and SIV, but there are several differences in the open reading frames in the central region. The homology of amino acid sequences of SIV$_{\text{MND}}$ is 57% in *pol* with HIV-1, HIV-2, SIV$_{\text{AGM}}$, and SIV$_{\text{MAC}}$ thus, SIV$_{\text{MND}}$ is equally distant to each virus including SIV$_{\text{AGM}}$.

From the genetic closeness between HIV-2, SIV$_{\text{MAC}}$, and SIV$_{\text{SM}}$, which was isolated from the sooty mangabey in the New England Primate Center, these three viruses can be considered as one virus group, HIV-2/SIV$_{\text{SM}}$. Thus, HIV-1 group, SIV$_{\text{AGM}}$ group, SIV$_{\text{MND}}$ group and HIV-2/SIV$_{\text{MAC}}$/SIV$_{\text{SM}}$ group are equally distant from each other, forming a regular tetrahedron in their relationship and do not overlap each other.

The phytogenic tree showing the evolutionary relationship among various primate lentiviruses was constructed after alignment of the amino acid sequences of *pol* genes. The tree clearly indicates primate lentiviruses, HIV and SIV evolved from a common ancestral virus, and SIV$_{\text{AGM}}$ and SIV$_{\text{MND}}$ diverged just prior to the divergence between HIV-1 and HIV-2/SIV$_{\text{MAC}}$/SIV$_{\text{SM}}$. We cannot explain why these four main groups of primate lentiviruses, SIV$_{\text{MND}}$, SIV$_{\text{AGM}}$, HIV-1, and HIV-2/SIV$_{\text{MAC}}$/SIV$_{\text{SM}}$ started to diverge at about the same time. The time of this divergence can be estimated to have occurred at least several hundred years ago, long before the spread of AIDS in humans in the 1980's. Thus, it can be concluded that SIV$_{\text{AGM}}$ and SIV$_{\text{MND}}$ are not responsible for the recent spread of AIDS in humans. However, it is possible that HIV-2, SIV$_{\text{MAC}}$, and SIV$_{\text{SM}}$ have been transmitted between monkeys and humans recently.

HTLV-I Infection and Adult Leukemia

Adult T cell leukemia (ATL) was first discovered and reported in Japan, where it has a high incidence in the southwestern region. The retrovirus HTLV-I (human T cell lymphotropic virus type I) is considered to be related to its etiology.

Typical ATL has the following features: (1) it occurs in adults; (2) tumor cells, i.e., ATL cells, resemble CD4 positive peripheral T-cells, having a notch in the nucleus and a tendency for lobulation; (3) skin lesions, hepatomegaly, splenomegaly, and lymphadenopathy are seen in a large percentage of patients, sometimes accompanied by pulmonary lesions or gastrointestinal lesions; (4) anemia is usually absent, and if present, is only mild; (5) hypercalcemia is not rare; and (6) patients are from Kyushu or its surrounding districts.

After the etiological involvement of HTLV-I was revealed, ATL was found to have diverse pathophysiological features which had not been suspected in the days when diagnosis of ATL was based on clinical features and cell markers. In diagnostically difficult cases, a definitive diagnosis can be made by checking the presence/absence of HTLV-I proviral DNA integrated into the DNA of tumor cells.

ATL can be divided into five types: (1) acute type; (2) chronic type; (3) smoldering type; (4) crisis type; and (5) lymphoma type. Of course, there are intermediate types which cannot be definitely allocated to any of these five types. ATL resists chemotherapy, and patients with acute and lymphoma types have quite a poor prognosis.

Infection with HTLV-I is a direct cause of ATL. However, infection with this virus can indirectly cause many other diseases via the induction of immunodeficiency, such as chronic lung diseases, opportunistic lung infections, cancer of other organs, monoclonal gammopathy, chronic renal failure, strongyloidiasis, nonspecific intractable dermatomycosis, and nonspecific lymph node swelling. The recently highlighted disease HAM (HTLV-I associated myelopathy) is thought to be synonymous with TSP (tropical spastic paraparesis). Clarification of the mechanism responsible for its onset is now an important theme.

Epidemiological studies have disclosed three routes of HTLV-I infection: (1) from mother to children; (2) from males to females; and (3) via blood transfusion. The data pertaining to HTLV-I infection by transfusion indicate that infection does not occur unless fresh lymphocytes are transfused. With regard to vertical transmission from mother to children, the lymphocytes contained in mother's milk are thought to carry HTLV-I. In the case of sexual transmission, transport is probably by lymphocytes in the semen. Some investigators claim that the virus can be transmitted by mosquitoes, but this has not been generally accepted.

The southwestern district of Japan has probably the world's highest incidence of HTLV-I infection. Besides, high incidence is also found in the Caribbean islands and countries surrounding the Caribbean Sea. Most of the ATL patients studied in the United States and the United Kingdom came from the Caribbean countries. The clinical features of ATL in the Caribbean area are similar to those in Japan, except that the age of onset of the disease is about 10 years younger in the former than in Japan. HTLV-I antibody-positive patients with malignant lymphoma have been reported in the United States, Italy, and Germany, although in small numbers. HTLV-I is said to have had its roots in Africa. However, no ATL case has yet been reported from Africa, and no comprehensive data on HTLV-carriers in Africa are available. In China, hardly any ATL patients have been reported, but considering the country's vast land and population, a large-scale survey is desirable. In Taiwan, the HTLV-I carrier rate is slightly higher in the northeastern region, and a small number of ATL patients have been diagnosed. In Korea, the HTLV-I carrier rate is as low as 0.03%; HTLV-I carriers have been found in Seoul and the surrounding areas, but no HTLV-I carriers have been found in Pusan and the Cheju Islands, even though both are close to Kyushu, Japan. Endemic areas of HTLV-I infection are now described from the Arctic to Papua New Guinea. Thus, a worldwide survey of ATL and HTLV-I infection is anticipated.

To prevent infection with HTLV-I in Japan all samples of donated blood have been subjected to HTLV-I antibody testing by the gelatin particle agglutination assay beginning in November 1986 on a nationwide scale. In order to prevent vertical transmission, HTLV-I seropositive mothers are now instructed to refrain from breast feeding in some local communities within the framework of a pilot study. This measure will also be implemented on a nationwide scale. Heating and freezing of milk are both known to be effective in eliminating the infectivity of HTLV-I, but these measures do not seem to be practical. Transmission between spouses is transmission between persons of the same generation; hence, there is hardly any risk of ATL onset even when this form of infection occurs. Therefore, what is required for the prevention of HTLV-I infection in the next generation is to have HTLV-I carrying mothers refrain from breast feeding. Experiments have disclosed that vaccination against HTLV-I is possible. However, there seems to be no necessity for vaccination.

Pharmacological Intervention of the Acquired Immunodeficiency Syndrome (AIDS)

Various steps of the human immunodeficiency virus (HIV) lifecycle can be considered as the possible targets for anti-AIDS chemotherapy. A brief summary of studies on the development of anti-HIV therapeutics using the MT-4 cell assay system follow.

Adsorption. HIV infection begins with the interaction of a viral *env* gene product gp120 with CD4 receptor on the cell surface. Soluble CD4 molecules generated by recombinant technology have inhibitory effect on viral adsorption, thus protecting cells from HIV infection. It was first discovered that sea-algal extract contained anti-HIV activity. The active principle of this extract was found to be a sulfated polysaccharide closely related to carrageenan. Further studies showed that various natural as well as synthetic sulfated polysaccharides were effective inhibitors for HIV infection. These substances clearly inhibit viral adsorption to the target cells and cell-to-cell infection. However, this blocking effect of viral adsorption by sulfated polysaccharides seemed to be nonspecific because they also blocked HTLV-I-induced cell fusion. Anti-HIV activity of sulfated polysaccharides appears to increase with the degree of sulfation. Also, higher activity is observed with the increase of molecular size of sulfated polysaccharide in the range of 1,000 to 20,000. Dextran sulfate has been used for 25 years in Japan as the drug for hyperlipidemia. Recently, efficacy of oral administration of this compound was evaluated in a Phase I study of AIDS and ARC patients. Unfortunately, no benefit has been achieved in terms of virological as well as immunological parameters. This is probably due to the fact that a blood concentration adequate to inhibit HIV cannot be reached by oral administration of dextran sulfate. However, dextran sulfate with anti-HIV activity cannot be administered intravenously because it elicits antibody formation and, moreover, it is degraded very rapidly in the blood. To overcome this problem, a different strategy was applied involving synthesis of novel sulfated polysaccharides. Among them, curdlan sulfate is expected to be used intravenously in the very near future for the treatment of AIDS and ARC patients since this is a beta-1,3-glucan and is much more stable in the blood than dextran sulfate (alpha-1,6 glucan). Glycyrrhizin, protein-bound polysaccharide (PSK) and an extract of lentinus edodes mycelia (LEM), which have been widely used in the patients with chronic hepatitis and malignant tumors in Japan, also block HIV adsorption to the CD4 receptors. Since they have almost no side effects, extensive clinical studies with these drugs will be evaluated, especially as related to protecting asymptomatic carriers from developing AIDS.

Reverse transcription. As is well known this is the most unique feature of retrovirus and therefore a number of reverse transcriptase inhibitors have been developed. Among them, azidothymidine (AZT) and 2,3-dideoxynucleoside analogues have appeared as potent and selective anti-HIV compounds *in vitro*. AZT has been approved as the drug of choice for treatment of AIDS in the United States and many other countries because it was shown to prolong the lifespan of patients and improve their immunological parameters. Using the same strategy we demonstrated the antiviral effect of 2,3-didehydro-2,3-dideoxythymidine. These dideoxynucleosides are assumed to act as substrate analogues after being converted to their triphosphate form before they can interact with reverse transcriptase and consequently cause viral DNA chain termination.

Viral DNA Synthesis and its Expression. Viral DNA replication, transcription into RNA, translation, and splicing of the viral RNA could also be good targets for anti-HIV chemotherapy. For this purpose, one of the most elegant approaches is the so-called antisense RNA or DNA strategy. In this approach complementary nucleotide oligomers are expected to hybridize to their target sequence in the viral genome resulting in inhibition of HIV infection. We selected the tRNA binding site and the splicing acceptor site of HIV as target sequences and synthesized RNA oligomers. These oligomers almost completely suppressed HIV infection at the concentration of about 5 ug/ml. Theoretically, it is also possible that antisense DNA and RNA can inhibit virus in cells chronically infected with HIV. Indeed, Matsukura et al., reported that this is the case. If so, this strategy will be very important for the treatment of individuals infected with HIV because the same effect cannot be expected with the inhibitors for viral adsorption or reverse transcriptase.

Other. Transcription of HIV mRNA is controlled by regulator gene products tat and rev. For the maturation of HIV, post-translational cleavage of gag-pol protein is essential. HIV *env* gene products also require post-translational modification; i.e., glycosylation of a large precursor protein and its cleavage into two smaller proteins, gp120 and gp41. The virus assembly and budding processes are, of course, essential to HIV for its spreading. Finally, it is suggested that some membrane protein, for example LFA-1, is involved in HIV infection and viral membrane fusion. Also, phosphorylation of the CD4 molecule after HIV binding has been shown to occur. All these steps of HIV replicative cycles can be targets of future anti-HIV drugs.

Treatment of Neutropenia in Patients With Acquired Immunodeficiency Syndrome With Granulocyte Colony-Stimulating Factor

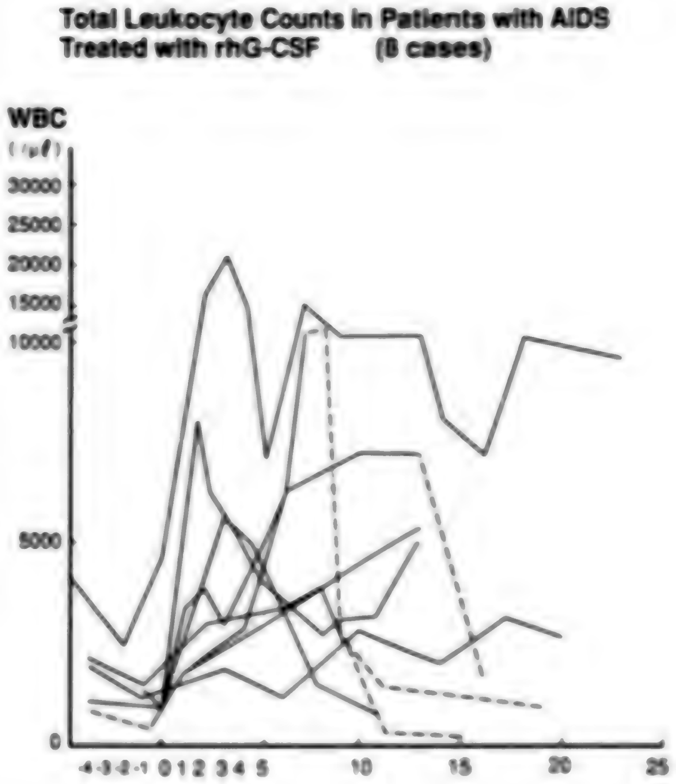
Neutropenia is often observed in patients with Acquired Immunodeficiency Syndrome (AIDS). It may contribute to the high incidence of opportunistic bacterial and fungal infections occurring in these patients. Human granulocyte colony-stimulating factor (G-CSF), a 19-KD glycoprotein hormone capable of stimulating neutrophilic granulocyte production and activating the functions of mature neutrophils, is now available for clinical use, due to successful large-scale production of the factor in a recombinant form. To establish the rationale for its clinical use, we have recently measured the G-CSF levels in serum (normal level: usually less than 30 pg/ml) using a newly established enzyme immunoassay method, and correlated those values to blood neutrophil counts and body temperature in four patients with AIDS.

Table 1 shows the serum G-CSF levels together with body temperature and blood in neutrophil counts in those four patients. In two patients (R.K. and T.K.), the relationship between blood neutrophil count and the G-CSF level appeared to be normal. Y.T. showed a very high G-CSF level (370 pg/ml) during a high fever episode but no neutrophil increase, suggesting a low response of granulocytic cells to G-CSF. In T.S. (39-year-old male), repeated examinations on different days revealed that the G-CSF level had never exceeded 30 pg/ml in the presence of marked neutropenia. Furthermore, G-CSF level showed a slight increase (76 pg/ml) during a febrile episode with a neutrophil count of 2,025/cm.

Table 1. Serum granulocyte colony-stimulating factor levels, body temperature, and blood neutrophil counts in patients with Acquired Immunodeficiency Syndrome

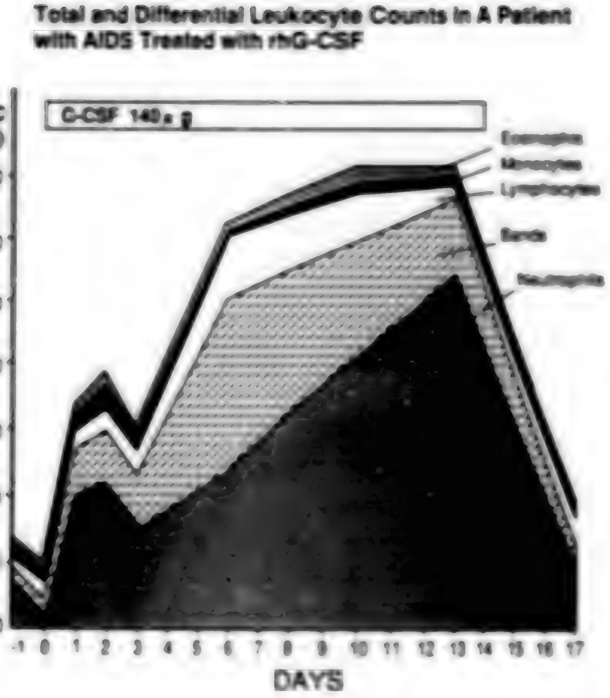
Patient	*Body Temperature*	*Blood Neutrophil Counts (/cmm)*	*Granulocyte Colony-Stimulating Factor Levels in Serum (pg/ml)*
T.S.	37.2	742	<30
	36.6	168	<30
	36.8	320	<30
	38.7	2,025	76
	36.7	871	<30
Y.T.	38.6	2,314	370
R.K.	36.7	1,804	<30
T.K.	35.7	3,591	91

As we had reported, serum G-CSF levels were increased in patients with neutropenia and this increase was inversely correlated to the neutrophil counts in peripheral blood. As shown in Table 1, in some AIDS patients, no increase of serum G-CSF level was observed even in the presence of neutropenia. This lack of an increase in the G-CSF level in response to neutropenia may be due to the infection of monocytes with AIDS virus, since G-CSF is usually produced by monocytes and macrophages except for the exceptional case.


Total Leukocyte Counts in Patients with AIDS Treated with rhG-CSF (8 cases)

AIDS patients with neutropenia and fever were treated with rG-CSF. Recombinant G-CSF (rG-CSF) made by *E. coli* and supplied from Kirin-Amgen Company was administered to seven patients daily for 7–25 days by the intravenous route. Administration of rG-CSF was started at 100–200 ug/m and increased to 400 ug according to the results of neutrophil counts. All those patients were confirmed to have AIDS by clinical syndromes and positive anti-HIV antibody test, and six out of seven cases showed neutropenia.

As shown in Fig. 1, all cases showed a [illegible]crease in white blood cell counts. This increase wa[illegible]ue to the increase in neutrophil counts.


Total and Differential Leukocyte Counts in A Patient with AIDS Treated with rhG-CSF

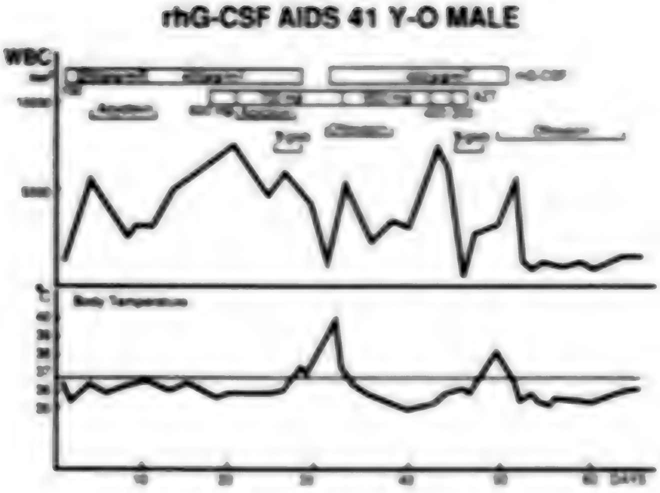
As demonstrated in a typical case shown in Fig. 2, a slight increase in lymphocytes was observed. This neutrophilic response was variable and seemed to be greater in patients with larger numbers of neutrophils before treatment.

The number of CD4 plus cells was examined before and two weeks after treatment with rG-CSF in five patients. No significant increase in CD4 plus cells was observed after treatment.

Secondary infections with known and unknown pathogens were present in five and two cases, respectively. Among five cases with a known pathogen, three responded to rG-CSF treatment with a marked reduction of body temperature. In one unresponsive case with disseminated herpes zoster, an increase in neutrophils after rG-CSF was insufficient due to the simultaneous usage of myelosuppressive agents. The other case was afebrile while suffering from oral candidiasis. In two febrile cases with an unknown pathogen, no or only slight clinical improvement was observed after rG-CSF treatment, although their peripheral blood neutrophil counts were increased. Fig. 3 shows a typical case who responded to rG-CSF administration with an increase in peripheral blood neutrophil counts and a reduction of body temperature.

No appreciable side effects were observed during the treatment with rG-CSF in any of the seven cases.

From above described results, rG-CSF is considered to be a useful drug for treatment of infections occurring in patients with AIDS.


rhG-CSF AIDS 41 Y-O MALE

FUTURE OBJECTIVES

On the basis of research results described above, future activities may be directed to the following research projects of highest priority:

1. Development of anti-HIV drugs with mechanisms other than AZT or other nucleoside derivatives as alternatives of these drugs, and prompt transfer to the clinical evaluation systems in the United States.
2. Further investigation of HIV gene expression regulation, and combined use of regulatory proteins as possible intervention with the HIV growth.
3. Development of vaccine candidates by conventional or recombinant DNA approaches, employing the conserved epitopes of env gene, regulatory proteins, or anti-idiotype antibodies.
4. Evaluation of possible animal models for therapeutics and vaccine developments; e.g., SIV or HIV infections in monkeys, transgenic mouse carrying CD4 receptor, FIV in cats, or MAIDS in mice.
5. Development of diagnostic procedures and reagents detecting HIV antigen or cDNA prior to seroconversion, either by new immunoassays or PCR, to ensure blood supply safety.
6. Epidemiology of HIV infections in risk groups in Japan or countries of Southeast Asia, in cooperation with WHO's global AIDS programs and individual national programs.
7. Studies on HTLV-1 infections: A) Confirmation procedure of diagnostic testing as in the case of HIV infections should be developed and evaluated. B) Establishment and installation of the screening and confirmation test systems to ensure the safety of blood transfusion and organ transplantation should be promoted. C) Basic studies on the factors, like transplantation antigen types, differentiating individuals to develop either ATL or HAM should be elucidated. D) Epidemiological intervention to reduce the HTLV transmission like discouragement of breastfeeding of HTLV-infected mothers should be evaluated quantitatively.

PROJECTS SUPPORTED BY THE PROGRAM

Investigator and Institution	*Title*
Shinji Harada Dept. of Virology Kumamoto University Medical School	Biological differences among plaque-cloned HIV isolates
Masakazu Hatanaka Institute for Virus Research Kyoto University	Mutations in transactivating factor of HIV
Toshio Hattori The Second Dept. of Internal Medicine Kumamoto University Medical School	Biochemical charecterizations of a neutralizing epitope of HIC, HTLV-III B strain.
Masanori Hayami Institute for Virus Research Kyoto University	Genetic analysis of SIV, AGM and SIV MND in comparison with HIV isolates Epidemiology of SIV infections in Africa
Yuzo Iwasaki Dept. of Neurological Sciences Tohoku University School of Medicine	Microglia reaction in HIV encephalopathy
Takashi Kitamura Dept. of Virology and Rickettsiology National Institute of Health	Epidemiological surveillance of AIDS in Japan Screening glycosidase inhibitors as possible HIV therapeutics.
Takeshi Kurata Dept. of Pathology National Institute of Health	Viral pathology of HIV associated with HHV-6 infection
Takashi Kurimura Dept. of Pathology Research Institute for Microbial Diseases	Biological properties of HIV isolates and their significance in the disease development
Masanao Miwa Institute of Medical Sciences University of Tsukuba	Prevention and control of AIDS in Japan
Takashi Okamoto Virology Division National Cancer Research Institute	Regulation of HIV gene expression
Mitsuhiro Osame The Third Dept. Faculty of Medicine Faculty of Medicine Kagoshima University	Immunological studies of HIV and HTLV-I dually infected hemophiliacs. HIV-1 etiology of HTLV-I associated myelopathy (HAM).
Kaoru Shimada Dept. of Infectious Disease Institute of Medical Science University of Tokyo	Treatment of cases of various stages of HIV infection by anti-HIV agents and chemotherapy of opportunistic infections
Yuichi Shiokawa School of Medicine Juntendo University	Epidemiological surveillance of AIDS in Japan and Southeast Asia
Kenji Soda Dept. of Public Health School of Medicine Yokohama City University	Prediction of future trends of AIDS and HIV infection in Japan

Fumimaro Takaku The Third Dept. of Internal Medicine Faculty of Medicine University of Tokyo	Treatment of HIV infection by granulocyte colony stimulating factor (G-CSF).
Kiyoshi Takatsuki The Third Dept. of Internal Medicine Kumamoto University Medical School	Hematological studies of adult T cell leukemia (ATL) caused by HTLV-1. Therapeutic use of monoclonal antibodies against HIV proteins.
Hideaki Tsuchie AIDS Research Center National Institute of Health	Genetic analysis of HIV isolates from cases of various stages of HIV infection.
Hiroshi Ushijima AIDS Research Center National Institute of Health	Effect of glucosidase inhibitors as HIV growth inhibitors.
Kaneo Yamada Dept. of Pediatrics School of Medicine St. Marianna University	Studies on HIV-infected hemophiliacs in Japan and possible counter measures.
Naoki Yamamoto Dept. of Virology and Parasitology School of Medicine Yamaguchi University	Immunological aspects of HTLV-1-associated myelopathy (HAM). Enhancement of HIV replication by tumor necrosis factor (TNF).

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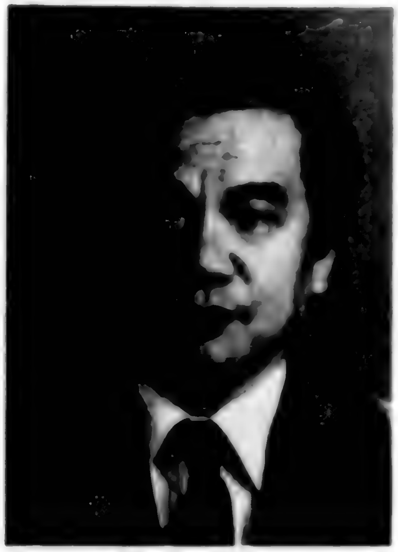

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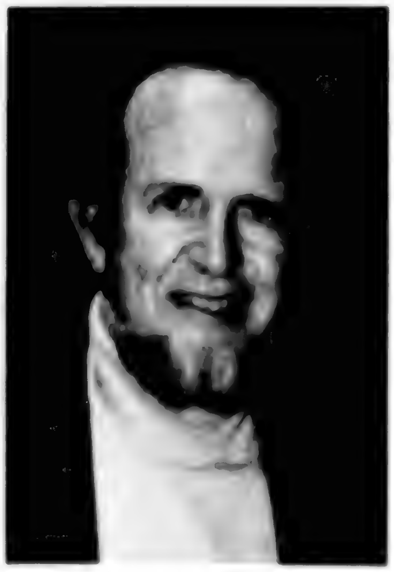
Profiles

U.S. CHOLERA AND RELATED DIARRHEAL DISEASES PANEL



Dr. R. Bradley Sack

Chairman (1983–1990)

Dr. Sack, a graduate of the University of Washington School of Medicine, has made a number of major contributions to the understanding of cholera at both the laboratory and clinical levels. His precise bacteriological studies were critical to establishing etiologic role of *E. coli* in cholera-like diarrheal diseases in cholera endemic areas. His major role in developing a canine model of cholera led to a number of increasingly sophisticated studies of the molecular biology of cholera, culminating in demonstration that the cholera toxin acts through stimulation of the adenylate cyclase enzyme in the intestinal mucosal cells. His early work on the effect of antimicrobial therapy in cholera was seminal in its effect on this field.

Dr. Sack continues to work closely with his colleagues in the diarrheal disease field, and his recent contributions have laid the groundwork for the current recommendations for the prophylaxis against traveler's diarrhea. During his tenure as Chairman, the Cholera Panels played a leading role in developing the first field trial of an orally administered cholera vaccine, based on scientific principles developed by the work of the Panels.

Dr. Nathaniel F. Pierce

Chairman (1977–1983)

Dr. Pierce has made seminal contributions to the understanding of both the physiology and treatment of cholera. He and his colleagues were the first to demonstrate, in a series of carefully controlled trials, that the oral administration of a solution containing glucose and electrolytes could restore metabolic balance in acutely ill cholera patients. This laid the groundwork for the current worldwide use of oral rehydration therapy. Dr. Pierce continues to work on dissemination of knowledge about oral rehydration therapy in his current position with WHO in Geneva.

Dr. Pierce was also one of the earliest scientists to recognize the role of intestinal secretory immunoglobulin A antibody in protection against cholera. His work in this field provided a solid background for the current approach to oral immunization in cholera.

Dr. Pierce's scientific career has been distinguished by his generous collaborations with coworkers throughout the world, and his unfailing willingness to share credit for scientific work which he has initiated. His Panel Chairmanship was distinguished by rapid increase in our understanding of the mechanism by which enterotoxigenic *E. coli* produce human disease, and an increased understanding of the protective immunologic mechanisms against *E. coli*.

Dr. John P. Craig

Chairman (1972–1977)

Dr. John P. Craig, a graduate of the Case Western Reserve University School of Medicine, played a seminal role in the description of the cholera enterotoxin. He was one of the early investigators to recognize the role of a specific enterotoxin in the pathophysiology of cholera, and was one of the first to isolate cholera enterotoxin, to produce the toxin in large quantities, and to demonstrate that, in the experimental animal, it produced all the signs and symptoms of human cholera. His ingenious development of a skin test for assay of the potency of the cholera toxin provided the gold standard for toxin assay for two decades, and contributed enormously to progress in this field.

Dr. Craig has always been a most enthusiastic collaborator with other investigators in this field. There are very few of them, Japanese or American, who are not indebted to Dr. Craig either for advice and consultation or for providing assay materials to further their research.

Dr. Craig's Chairmanship was marked by a period of rapid advances in understanding of the biochemical mechanisms by which the cholera enterotoxin caused intestinal fluid secretion, as well as increasingly precise delineation of the protective immunologic mechanisms against cholera.

JAPANESE CHOLERA AND RELATED DIARRHEAL DISEASES PANEL

Dr. Yoshifumi Takeda

Chairman (1988–present)

Dr. Takeda graduated from the School of Medicine, Osaka University in 1960, and successively held the posts of professor at the Institute of Medical Science, professor at the University of Tokyo (1983–1989), and professor at Kyoto University (1987–). He has been Chairman of Japanese Cholera Panel since 1988. In 1988, he sponsored the 24th Joint Cholera Conference held in Tokyo, Japan. The Chairman of the U.S. Cholera Panel during his term of office was Dr. R. Bradley Sack.

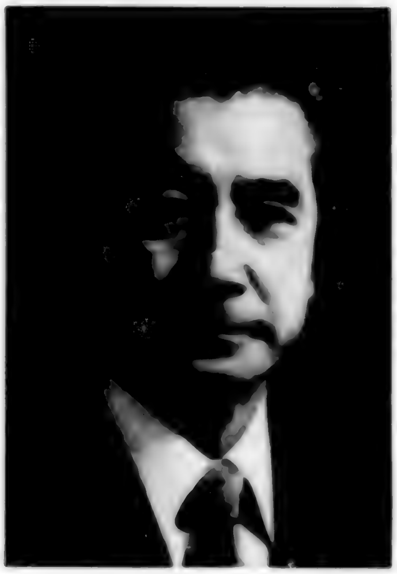
Dr. Takeda has been engaged in many studies on toxins of diarrheagenic bacteria, such as *Vibrio parahaemolyticus*, *V. cholerae* 01, enterotoxigenic *Escherichia coli* (ETEC), and enterohemorrhagic *E. coli* (EHEC), and has been a leader in the field. Dr. Takeda is a cosmopolitan who stresses the importance of cooperation in research between various countries and organizations such as the WHO and ICDDR-B, and also encourages the development of young researchers in Japan. He is a highly respected international leader in research related to bacterial diarrhea in Japan.

Dr. Yutaka Zinnaka

Chairman (1987)

Dr. Yutaka Zinnaka graduated from the Faculty of Medicine, Kyushu University in 1951, and successively held the posts of professor at National Defense Medical College (1975–1986), and Professor at Toho University (1987–1988). He chaired the Japanese Cholera Panel for 1 year in 1987. The Chairman of the U.S. Cholera Panel during his term of office was Dr. R. Bradley Sack.

Of Dr. Zinnaka's research achievements, ultrastructural analysis of cholera toxin subunits by electron microscopy and analysis of toxins of NAG vibrio are particularly well known. Needless to say, Dr. Zinnaka's greatest interest was in cholera research, but he had other interests which included his becoming familiar with the culture, history, art, and music of various countries, which he accomplished by visiting the Silk Road in China, the Sistine Chapel in Rome, and museums and art galleries in various countries of the world. Dr. Zinnaka died on June 15, 1988.



Dr. Nobuya Ohtomo

Chairman (1985–1986)

Dr. Ohtomo graduated from the Faculty of Medicine, Kyushu University in 1948, and held the post of Managing Director, the Chemo-Sero-Therapeutic Research Institute (1982–1986). Since 1987, he has been Director of the Oita Prefectural Institute of Health and Environment. The period of his chairmanship of the Japanese Cholera Panel was from 1985 to 1986 at which time Dr. R. Bradley Sack was Chairman of the U.S. Cholera Panel. During this period, he sponsored the 22d Joint Cholera Conference held in Toyama, Japan, in 1986. After the Conference, the participants started on a laborious excursion, organized by Dr. Ohtomo, going over Tateyama (a height of 3,015m) and crossing the Kurobe Valley and a dammed lake during which they enjoyed the remaining snow and realized their own youthfulness anew while being refreshed by the mysterious atmosphere in Kurobe Valley.

Dr. Ohtomo is currently the leader of cholera toxin research in Japan.

Dr. Shogo Kuwahara

Chairman (1980–1984)

Dr. Kuwahara graduated from the School of Medicine, Tokyo Imperial University in 1943, and held the post of professor at Toho University from 1954–1986. Since 1969, he has been Chairman of the Boarding Committee, Toho University. For 5 years, 1980–1985, Dr. Kuwahara served as Chairman of the Japanese Cholera Panel. He sponsored the Joint Cholera Panels Conference held in Japan in Gifu (1980) and in Kurashiki (1982) during his tenure. Chairmen of the U.S. Cholera Panel during his term of office were Dr. Nathaniel F. Pierce and Dr. R. Bradley Sack.

The major theme of his research during his term of office as the Chairman of Japanese Cholera Panel was the prevention of epidemics by El tor cholera because prevalence of cholera was then expanding from its endemic areas to the temperate regions. For this reason, Dr. Kuwahara encouraged research on cholera vaccine development in Japan. He also strived for the introduction of molecular genetics into research with enterotoxigenic *Escherichia coli* (ETEC), which causes travelers' diarrhea. He discovered self-transmissible drug-resistance (R) plasmids of *Vibrio cholerae* 01 and has been the major proponent for research on bacterial virulence genes in Japan.

Dr. Kenji Takeya

Chairman (1978–1979)

Dr. Takeya graduated from the Faculty of Medicine, Kyushu Imperial University in 1944, and successively held the posts of professor at Kyushu University (1963–1982), and president of Kyushu University (1975–1982). He served as Chairman of the Japanese Cholera Panel for 2 years from 1978 to 1979 and sponsored the Joint Cholera Panels Conference at Karatsu, Japan, in 1978. The Chairman of U.S. Cholera Panel when Dr. Takeya took office was Dr. Nathaniel Pierce.

While engaged in research on *Vibrio cholerae* 01 biotype El tor, Dr. Takeya discovered a new prophage which is now named k (kappa) phage and developed a prophage typing method for *V. cholerae* 01 biotype El tor. Dr. Takeya died on January 22, 1982.

Dr. Hideo Fukumi

Chairman (1965–1977)

Dr. Fukumi graduated from the School of Medicine, Tokyo Imperial University in 1938, and successively held the posts of professor at the Institute of Tropical Medicine, Nagasaki University (1963–1969) and Director-General of the National Institute of Health (1977–1980). He is an Honorary Member of the National Institute of Health at present. Dr. Fukumi served as Chairman of Japanese Cholera Panel for 13 years from 1965 to 1977. He sponsored six Joint Cholera Panels Conferences which were held in Hakone (1966), Unzen (1968), Hakone (1970), Tokyo (1972), Kyoto (1974), and Sapporo (1976), during his term of office. As the first Chairman of Japanese Cholera Panel, Dr. Fukumi worked tirelessly for the development of U.S.-Japan Cholera Panels in cooperation with Dr. Charles C. J. Carpenter, Chairman of the U.S. Cholera Panel and his successor, John P. Craig.

When the 7th cholera pandemic due to *Vibrio cholerae* 01 biotype El tor developed, Dr. Fukumi promoted cholera vaccine field trials in the Philippines by quickly making cooperative arrangements between Japan, the Philippines, and the WHO. By evaluating the experimental results, he was able to formulate quarantine policies based upon his elucidation of the limited effectiveness of the cholera vaccines available in those days. Dr. Fukumi is the father of cholera research in Japan and has acted as its director and also as a promoter for research on cholera toxin.

GUIDELINES

CHOLERA AND RELATED DIARRHEAL DISEASES PANELS
United States-Japan Cooperative Medical Science Program

All research on cholera as well as cholera-like diseases (e.g., enterotoxigenic *Escherichia coli*) is relevant to the panels' interest. The following areas have been assigned a high priority:

1. *Methods of immunization*. Improvements of whole cell vaccines, development of effective adjuvants, isolation and purification of protective antigens, and development of an effective toxoid;
2. *Pathogenesis and treatment*. Purification of responsible exotoxin, studies of mechanisms of action of this toxin, and development of a pharmacologic means of rapidly blocking the action of the toxin; related investigations of basic mechanisms of secretion and absorption of water and electrolytes in the small intestines;
3. *Microbiologic investigations*. Studies of those properties of *Vibrio cholerae* which are essential to its multiplication in the gut of the susceptible host, and of those properties of the host which render him vulnerable to *V. cholerae* infection.
4. *Vibrio ecology*. Investigations of factors in the environment (e.g., food and water) outside the human host which influence population, distribution, growth rate and virulence of cholera vibrios.

Cholera-like diseases are defined as those in which the relationship between bacteria living in the lumen of the gut and a human host is such that the resulting interaction will not cause (1) tissue destruction, (2) symptoms of toxicity, or (3) bloody diarrhea. If the diarrheal disease becomes life-threatening, it is on the basis of fluid loss.

U.S. Cholera and Related Diarrheal Diseases Panel

PROGRESS IN PRIORITY AREAS

At the beginning of this 5-year period, the scope of research of the panel was enlarged to include other bacterial diarrheal pathogens, including invasive pathogens. In 1987 the official name of the Cholera Panel was changed to include "Related Diarrheal Diseases," which more accurately reflected the interests of the panel and the investigators.

During this period, major new advances in biomedical technology occurred, such as the technologies involving monoclonal antibodies, genetic engineering, and DNA probes, which had a major influence on work being done in the field of bacterial, diarrheagenic diseases. As a result of this, it became possible to study in more detail, the molecular aspects of virulence of these organisms.

Because of the expanded scope of the panel, the report will be presented according to individual pathogens, of which there are now about 20. The major emphasis, however, continues to be on *Vibrio cholerae* and means whereby cholera may be controlled.

1. *Vibrio cholerae*:

Vaccine development continues to be the major focus of this work. Studies of pathogenesis, secretory immunity, and bacterial genetics all have as one of their ultimate objectives, the stimulation of the human immune system safely and efficiently so that a practical vaccine might eventually be realized. Much progress has been made in this area, and a large field trial of a candidate cholera vaccine has been completed.

a. Immunologic studies:

The mucosal immune system has been extensively studied, once this was shown to be the major component in conferring protective immunity against *V. cholerae*. Live orally administered vibrios which colonize the small intestine well have been found to be the most effective antigens. This is thought to be the result of improved sampling of live organisms by the M cells in the intestinal mucosa. The ability of vibrios to produce whole toxin or toxin subunits was of lesser importance than their ability to colonize. Anti-cholera antibodies in maternal milk of animals, while providing protection against cholera when being fed to susceptible young animals, also interfered with the development of active immunity by those young animals. Such antibodies (anti-toxin, anti-outer membrane protein antigens) can also be produced in cows by hyperimmunization with the appropriate antigens; these antibodies in a milk concentrate are relatively protease-resistant, and therefore are active when fed orally.

Antisera against TCP pili are also highly protective in the infant mouse model. The specific epitopes of the pilus involved in colonization are being defined using antisera against synthesized specific pilus peptides.

b. Studies of pathogenesis:

In mice, resistance to the secretory effect of cholera toxin is found to be under the genetic control of the major histocompatibility complex (H-2). This has been found to be due to the decreased numbers of receptors (gangliosides) on the enterocytes of resistant mice.

When grown under the conditions of iron deprivation, *V. cholerae* produce unique outer membrane proteins. Monoclonal antibodies to these outer membrane proteins have been produced and the possibility that these proteins are protective antigens is under study.

The role of motility as a virulence property is again being examined. Non-motile, but flagellated mutants are not virulent in certain animal models. Purification of the sheathed flagellum is being attempted since it contains unique antigens that may be protective.

Three types of morphologically and serologically distinct fimbria have now been identified in *V. cholerae*. While the TCP pili have been well characterized, the other two are currently under investigation.

Epitopes of the cholera toxin family have been defined using monoclonal antibodies. At least three distinct cholera toxins have been identified which differ in a small number of amino acid residues. These small differences influence their neutralization by antibodies, and thus may be important in defining antigens in vaccine development.

Adherence of *V. cholerae* based on hemagglutinins, both cell associated and soluble is also being studied. Mutant strains lacking cell-associated hemagglutinins showed decrease ability to adhere to test cells.

Studies defining the role of prostaglandin E in the molecular events leading to intestinal fluid secretion caused by cholera toxin are in progress. It is postulated that prostaglandin E may contribute significantly to the total cyclic AMP that accumulates in cells following cholera toxin exposure.

Sodium channel inhibitors were detected in culture filtrates of *V. cholerae* 01 and non-01. These tetrodotoxin-like substances are being investigated as to their possible role as virulence factors.

c. Genetic Studies

Genetic studies have defined the regulatory genes for the production of cholera toxin, as well as for other virulence factors, such as pili. All *V. cholerae*, including non-toxigenic strains, possess the regulatory *toxR* gene. This gene has been sequenced and its gene product, a transmembrane protein, identified. The *toxR* coordinately regulates the genes responsible for cholera toxin production and for TCP pilus production, as well as 17 genes that encode additional virulence factors. The genes encoding for cholera toxin (*ctx*) and the major subunit of the TCP pilus (*tcpA*) have been cloned and sequenced. This pilus, called TCP because it is *t*oxin *c*o-regulated, has areas of homology with pili present on other bacterial species, such as *pseudomonas* and *gonococcus*.

The recombination and replication activities of RS1, a repetitive sequence associated with the cholera toxin genetic element (*ctxAB*) are also being studied. It appears that RS1 encode a site-specific recombination system capable of highly efficient conversion of nontoxigenic strains of *V. cholerae* into toxigenic ones. Such interconversion apparently occurs naturally in the Gulf Coast of the United States, based on the co-existence of toxigenic and nontoxigenic strains that appear isogenic, except for the presence or absence of the *ctx* element. The *ctx* gene can gain entry into nontoxigenic strains experimentally by either conjugation, transduction, or transformation, although how this occurs in nature is unknown. Thus, it would seem that inactivation of this RS1-mediated recombination/replication system might be important in vaccine development in order to reduce the likelihood of vaccine strains reverting to toxicity by contact with bacteria carrying the *ctx* gene.

Both classical and El tor strains require TCP pilus expression for colonization and initiation of disease in animals and human studies. Although the classical and El tor TCP pilis are not identical, they are about 80% homologous, and retain nearly identical antigenic properties.

d. Volunteer Studies:

A number of studies have been done, using candidate oral vaccine strains produced by genetically altering and attenuating known virulent strains of *V. cholerae*. Initial vaccine candidates produced solid protection, even though they did not contain any cholera toxin genes. Diarrhea developed, however, in about 50% of volunteers given these strains, thus limiting their potential usefulness.

Additional candidate vaccines became available (which were devoid of any known cytotoxic activity, felt to be partially responsible for the diarrhea in earlier studies) which did not cause diarrhea in volunteers, but which were highly immunogenic and protective. Such a strain is now available (CVD 103, A−B+) that contains a gene for mercury resistance which makes it readily identifiable; it is being tested in volunteers living in endemic cholera areas. It is hoped that a single dose of this live-attenuated vaccine may confer a high degree of longlasting immunity.

Volunteer studies have also been done with genetically engineered strains that further define the requirements for an optimal vaccine strain. Intense colonization of the small bowel was a requirement for a brisk antibody response, and strains which lacked the newly described TCP pilus were unable to colonize well. Thus, the presence of the pilus was felt to be an important component of candidate cholera vaccine strains.

e. Vaccine field studies:

A large double-blind, placebo-controlled field test of two candidate oral non-living cholera vaccines has been carried out in 63,000 persons in Bangladesh, with a completed 3-year followup. One vaccine was made only from whole killed cholera vibrios (WC), while the other one contained in addition the B subunit of the cholera toxin (WCB). Three doses were given; no side effects were detected. At 6-months followup, the WC vaccine provided 58% protection, while the WCB vaccine gave 85% protection (a significant difference) as compared to placebo. At 1 year, the protection rates were not significantly different (53% for WC and 62% for WCB). At 3 years, the protective efficacy remained equal for the two vaccines (50%–52%). There was, however, a considerable difference in vaccine efficacy depending on the age at immunization: older children and adults had about a 65% protection, whereas for children between the ages of 2–5 years, it was only about 25%. The vaccine effect was also sustained at a higher level in older persons.

The WCB was also found to be highly protective (75%) against diarrhea caused by heat-labile enterotoxin-producing *E. coli*, whereas the WC vaccine had no protective effect. The protection lasted, however, only for about 3 months following the immunization. The protection was clearly due to antitoxic immunity produced by the cholera toxin B subunit component of the vaccine, which is immunologically similar to the heat-labile enterotoxin of *E. coli*.

These studies indicate that an oral nonliving crude whole cell vaccine, which is inexpensive and free of side effects, can provide significant longlasting protection in older children and adults living in a cholera endemic area, and therefore could be a useful public health tool. Because this vaccine needs to be given on multiple occasions, and because the efficacy is less than optimal, there is clearly a need to continue to develop an optimal live oral vaccine. Ideally, this live vaccine would produce all the appropriate protective antigens (including the TCP pilus, flagellar antigens, outer membrane proteins, cell-associated hemagglutenins, in addition to LPS and the B subunit of the cholera toxin), would be free of any side effects, could be given in a single dose, and would provide longlasting protection.

f. Studies of epidemiology and ecology:

Clinical cases of cholera due to *V. cholerae* 01 continue to occur in the United States. Essentially, all have been traced to consuming vibrio-contaminated crabs, shrimp, and oysters from the Gulf of Mexico, which is known to be an endemic focus of such infected shellfish.

A number of new techniques have been developed to detect vibrios in the environment. These tests have become important because of the demonstration of viable, nonculturable *V. cholerae* existing in the environment. These organisms have been shown to be viable by inoculation into both animal models and into human volunteers. In the latter studies, viable vibrios were cultured from the stools following challenge with nonculturable *V. cholerae* 01, although no clinical illness was produced. Techniques used to identify these organisms include immunofluorescence using monoclonal antibodies, enzyme assays, and DNA extraction procedures. Using these detection methods to sample water in Bangladesh, *V. cholerae* were found in considerably greater concentrations than by traditional cultural methods.

Vibrios were also found to adhere to the chitin of copepods and to plankton. They had previously been found to adhere to duckweed and larger aquatic plants, such as water hyacinth in Bangladesh. Clearly, the maintenance of vibrios in the aquatic environment by these different mechanisms may be of great importance in the natural history of the disease.

2. *Enterotoxigenic Escherichia coli* (ETEC)

Work on ETEC has generally paralleled similar work done with *V. cholera*, because of the many similarities between the two organisms. Because of the greater diversity of antigenic structure in ETEC, however, the progress in vaccine development has been considerably slower.

a. Enterotoxins:

1. **Heat-labile enterotoxin (LT).** This toxin, which is closely related to cholera toxin, has been extensively studied in terms of its epitopes, using monoclonal antibodies. There are multiple epitopes within the GM1 binding domain of the LT enterotoxins; a single amino acid substitution has significant effects on the reactivity of the toxin. It has been found that the B subunits of human and porcine strains of ETEC differ by only 4 of their 103 amino acids.

The LT is contained within the periplasmic space of the ETEC, rather than being transported extracellularly. The mechanisms whereby LT is released from the bacterial cells has been studied. Bile salts have been found to cause such release, but without causing cell lysis. It is postulated that this may be important in the milieu of the small intestine.

Gene-fusion techniques have resulted in the construction of genes which control both the production of the B subunit of LT and the heat-stable toxin (ST) of ETEC, resulting in the production of a hybrid molecule which retains the biological properties of both of the individual molecules. Such a hybrid molecule could potentially be a useful immunogen for both enterotoxins.

2. **Heat Stable Enterotoxins (ST's).** The synthesis and transport of the STa molecule out of the bacterial cell has been studied extensively. The molecule is initially synthesized as a 72 amino acid precursor, which is processed to eventually yield the final extracellular, active 18 or 19 amino acid ST.

Monoclonal antibodies have been developed which are used in a quantitative ELISA assay; these antibodies are also neutralizing, and do not cross-react with the ST of *Yersinia enterocolitica*.

The binding of STa to the enterocyte has been the subject of much study and much controversy over the past several years. Three molecular forms of the ST receptor have been studied, and the receptor is thought to be separate from the particulate guanylate cyclase. Both molecules have been purified separately. The binding is reversible, unlike that of LT or cholera toxin. The ST of ETEC and that of *Y. enterocolitica* compete for the same receptors. The jejunal mucosa of young animals was found to inactivate STa more slowly than that of older animals, suggesting a possible explanation of the described greater severity of ST-mediated ETEC disease in young animals, as compared to older ones. STa has been completely synthesized, and milligram quantities of bioactive toxin are now available.

The genetic material controlling STb was found to reside on a defective transposon. Only low amounts of toxin are usually found in culture filtrates. The toxin has now been purified, and found to be a polypeptide chain of 48 amino acids. The species specificity of this toxin may not be as narrow as originally thought; since it is sensitive to trypsin degradation, it may not be biologically active in some animal systems unless the intestine is washed with a soybean trypsin inhibitor. If this is done, STb can be assayed in other animal models, in addition to the pig.

b. Colonization Factor Antigens (CFA's).

New CFA's continue to be described. At least nine such antigens have been described in the last several years. Many, but not all of them are structural proteins, such as pili. All are antigenically distinct; some are associated with only one or a few serotypes of ETEC, while others are found in a large variety of serotypes. They are controlled by genetic material usually residing on plasmids. To date only one, CFA I, has been cloned.

c. Vaccines:

In animal models, the protective antigens of ETEC have been found to consist of 1) the 0 antigen, which is highly protective, but type-specific, 2) the B subunit of LT, which is less protective, but cross-reactive to all strains of LT-producing ETEC, and 3) colonization factor antigens, which are also highly protective, but type specific.

In volunteer studies, two candidate oral ETEC vaccines have been studied. Using a live ETEC vaccine strain which produced CFA I, but not enterotoxins, the immune response to the CFA I antigen was poor. Using a nonliving ETEC strain treated with colicin E2, a potent endonuclease which does not alter the integrity of the bacterial cells, and given in two doses, protection was achieved against homologous and heterologous challenge of living organisms. A secretory antibody IgA response to CFA I was seen, but it was not clear which antigens conferred the protection.

Passive immunity was achieved in a group of volunteers fed a milk immunoglobulin concentrate, prepared by immunizing cows with an array of ETEC antigens. The preparation was given three times a day and at the time of challenge with a virulent ETEC strain. Complete protection against diarrheal disease was achieved, demonstrating the efficacy of locally available antibody in the gastrointestinal tract.

3. Enteropathogenic Serotypes of *Escherichia coli* (EPEC):

During the past 5 years, new assays have become available to recognize these strains of *E. coli* which have been incriminated epidemiologically with diarrheal disease in infants. A tissue culture assay, using HEp-2 cell adherence, was found to distinguish most classical EPEC strains from non-EPEC strains. In the course of these studies, three distinct types of adherence patterns have been identified: a localized form, which correlated best with the identification of most EPEC serotypes; a diffuse type, which was not clearly correlated with serotype identification; and an auto-agglutinable type, which also did not correspond to specific serotype identification. The genetic material controlling these three types of adhesiveness was found to be located in plasmids, and DNA probes were developed, which made the identification of these strains more widely available. Using both the DNA probe assays and the HEp-2 assays, several epidemiological studies have been done correlating these strains with independent serotype identification as well as with diarrheal disease in children. Clearly, the localized form of adherence correlates well with diarrheal disease in children, whereas the diffuse type is generally not associated with illness. The auto-agglutinable type has been found to be correlated with diarrhea, often persistent diarrhea in children.

Other assays have been developed using human colon carcinoma cell lines, which seem to correlate well with the HEp-2 assays.

More recent genetic studies have shown that two adhesive properties of some EPEC strains could be clearly differentiated.

One, plasmid mediated, encoded the ability of the bacteria to adhere non-intimately to brush borders of enterocytes, whereas the second, chromosomal in location, encoded the ability of the bacteria to attach more intimately and efface the brush borders, thus mimicking the pathology demonstrated in animal and human disease with these organisms. A DNA probe for recognizing these chromosomal genes has been developed and shown to recognize these genes not only in classical EPEC strains, but also in RDEC-1 (rabbit) *E. coli* and in human enterohemorrhagic strains of *E. coli*.

The genetic determinants controlling diffuse adhesion have also been further studied. Genetic material located in a plasmid was found to encode for not only the diffuse adherence property, but also mannose-resistant hemagglutination and fimbria expression. The receptor for the fimbria was found to be the Dr blood group antigen, similar to that shown to be the receptor for the pili of uropathogenic strains of *E. coli*.

As a result of the new information on adherence properties and their genetic control, the classification of this broad group of *E. coli* is not clear-cut at the present. The term "enteroadherent" has been suggested to designate these strains, while the term "enteropathogenic" designates the well-recognized serotypes. Clearly, there is considerable overlap between these two classifications. It is probably best to characterize such an *E. coli* strain both by its serotype and by its pattern of adherence and/or adherence genes.

4. Enterohemorrhagic *Escherichia coli* (EHEC)

The past 5 years have seen the recognition of an entirely new group of diarrheagenic *E. coli*, those associated with hemorrhagic colitis, and the development of the hemolytic uremic syndrome.

These strains have been found to produce high levels of a Shiga-like toxin (also called Vero toxin), which is controlled by toxin-converting bacteriophages. To date, at least three separate Shiga-like toxins (SLT's) have been identified. The genes controlling the toxin production have been cloned and sequenced, and the amino acid structure of the toxins has been determined. The SLT's are similar in subunit structure to both the family of cholera entertoxins and to Shiga toxin, in that they have five binding subunits and one active subunit. The structure of SLT I is identical to that of Shiga toxin, except for one amino acid difference. The structure of SLT II has identical A subunits to SLT I, but differs in the B subunit structure, and therefore in antigenicity. The mechanism of action of these toxins is on the villous, rather than the crypt cells, and does not involve cyclase activation. Rather the toxins interfere with protein synthesis by a mechanism identical to that of Ricin, a plant toxin, with which they share homology in their A subunits. The toxins bind to a Gb3 receptor, as well as to the P1 glycoprotein blood group antigen.

Using a number of animal models, it has been shown that the SLT's have a direct toxic effect on vascular endothelial cells, as well as kidney cells, both of which have appropriate receptors for binding the toxin.

The major *E. coli* serotype which produces these toxins is 0157:H7, which has been shown by the multilocus enzyme approach, to be a single clonal type, unrelated to other 0157 strains or to Shigella strains. Additional serotypes of *E. coli* have also been found to produce the toxin in high amounts, but these are not as widely implicated in disease outbreaks.

EHEC are known to be responsible for both sporadic and outbreak-related cases of both hemorrhagic colitis and the hemolytic uremic syndrome. The latter is now thought to be the direct result of absorbed SLT on the vascular endothelium and kidneys.

5. Shigella:

The Shiga toxin has been of major interest during the past 5 years, both because of its possible role in Shigellosis, and because of its near identity with Shiga-like toxin I of *E. coli*. The Shiga toxin has been purified, and as stated above, consists of five binding and one active subunit, and has mechanism of action identical to the SLT's and Ricin.

The binding of Shiga toxin to the P1 blood group determinant which is found in hydatid cysts, has led to simpler methods for purification of the toxin, and to development of simplified assays.

Shiga toxin is produced only by *S. dysenteriae* 1, and not by other Shigella strains. It can be identified in the stools of patients with diarrheal disease due to this organism. The toxin, however, has not been clearly implicated in the dysentery illness, which clinically is quite different from the hemorrhagic colitis caused by EHEC. Furthermore, the hemolytic uremic syndrome seen following Shigellosis is frequently produced by other strains of Shigella that do not make this toxin.

The Shiga toxin has been found to be a strong adjuvant in the intestinal tract, in a way similar to cholera toxin. The local immunologic response to Shigella infection is initiated by the M cells which take up the Shigella organisms, thus, also providing an entry point for the establishment of invasive disease.

All Shigella strains and invasive strains of *E. coli* possess a large molecular weight plasmid which codes for proteins involved in the invasion process. These protein antigens, called ipa antigens are of interest because 1) they induce antibodies that may be cross-protective against these organisms, and 2) these antibodies could be useful in devising diagnostic reagents for the recognition of these organisms.

Clinical studies have demonstrated that both serum and secretory antibodies are produced against the ipa antigens as a result of Shigella infection; titres are much higher in adults than in children. It is not yet known, however, whether these antibodies are protective. These antigens are of obvious interest in the development of Shigella vaccines.

Epidemiologic studies in Peru have shown that household transmission of Shigella organisms both within and between adjacent homes is relatively infrequent. There is, however, a high rate of asymptomatic carriage among children in the household (approximately 5%), with organisms introduced from external sources into the homes.

There continues to be considerable interest in the development of oral Shigella vaccines, however no candidate vaccines are yet ready for field trials.

6. Other Diarrheagenic Bacteria:

a. *Vibrio parahemolyticus*

The genes controlling the thermostable direct hemolysin (the Kanagawa phenomenon-associated hemolysin) have been cloned and sequenced, and a DNA probe developed for diagnostic purposes. The probe is more sensitive than the use of Wagattsuma agar. Homologous genes are also present in *Vibrio hollisae*.

The genes controlling this hemolysin have more recently been found to be duplicated in hemolytic strains, but the two copies are not identical. Studies are in progress, with the collaboration of Japanese investigators, to determine whether one or both copies of the gene are necessary for hemolysis and/or pathogenicity.

Some strains, both Kanagawa positive and negative, have been found to produce a toxin which elongates CHO cells, reacts with anti-cholera toxin antibodies in an ELISA assay, but is not neutralized by the cholera antisera. DNA probe studies indicate some homology between this toxin and the cholera family of toxins. Whether this toxin is important in pathogenesis of diarrhea is not known.

b. *Vibrio cholerae non 01*

Challenge studies were done in volunteers using three different strains which were known not to produce cholera toxin. One of the strains produced significant diarrhea. The mechanism responsible for the diarrhea is not known, but this virulent organism did produce an ST toxin.

c. *Yersinia enterocolitica*

The chemical structure of the ST toxin produced has been elucidated. It consists of a single peptide of 27 amino acids and aggregates with itself during purification, initially giving the impression that multiple species of the toxin existed. The ST of *Y. enterocolitica* has a similar amino acid structure in part of its molecule to that of *E. coli*. The ST's of this organism and *E. coli* bind to the same intestinal receptors, but only the one produced by *Y. enterocolitica* activates nonintestinal guanylate cyclases.

Immunologic cross-reactivity between the ST's of *Y. enterocolitica* and *E. coli* varied, depending upon the coupling agents used to produce the antibodies. Some monoclonal antibodies produced to the ST of *E. coli* do not react with the ST of *Y. enterocolitica*.

d. *Salmonella*

Approximately 50% of strains of *S. typhimurium* were found to produce small amounts of a cholera-like enterotoxins, as assayed by an ELISA method. Monoclonal antibodies reacted both to the enterotoxins of these *Salmonella* strains and to the enterotoxin of *V. cholerae*.

The gene responsible for this cytotonic enterotoxin has been cloned into *E. coli* and the toxin has been partially purified. Several peptides from this toxin preparation have been identified that clearly react with cholera antitoxin in Western blots. It is not known, however, whether this toxin is important in the pathogenesis of the diarrhea produced by these organisms.

e. *Campylobacter pylori*

Genetic studies have focused on the development of systems that would allow the molecular determinants of pathogenicity to be characterized. A series of shuttle and suicide vector plasmids were constructed in order to introduce cloned genes into *Campylobacter* organisms by conjugation with *E. coli* donors. A method was also developed to transform intact *Campylobacter* cells with shuttle plasmid DNA using high voltage electroporation. With the use of these methods, genetic libraries of *Campylobacter* DNA in recombinant plasmids have been established.

f. *Clostridium difficile*

The two toxins produced by these organism, A and B, have been purified and characterized, and their binding properties studied. Immunologic assays have been developed for identifying the presence of the toxins in stools. Monoclonal antibodies show some cross reactions between toxins A and B, indicating that these toxins share a low number of epitopes.

g. *Clostridium perfringens*

The enterotoxin produced by these organisms is responsible for symptoms of "food poisoning." Monoclonal antibodies produced against the toxin are neutralizing and inhibited specific binding to intestinal brush border membranes. Receptor proteins in the brush border membranes have now been identified. Immunologic assays have been developed for identification of the enterotoxin in stools, such as the latex bead assay, which are more reliable indicators of illness produced by these organisms than are standard microbiological methods.

h. *Staphylococcus aureus*

Staphylococcal enterotoxin B induces multiple suppressor lymphocyte populations. Immunosuppression to multiple antigens occurs, not only to the homologous antigen. These findings may be of general importance in studying the immune response to antigens of infectious agents.

The amino acid sequences of enterotoxins A,B, and C have been found to show a 13 amino acid residue sequence with significant homology among the three enterotoxins. Some homology is also shown with the pyrogenic exotoxin of *Streptococcus pyogenes*.

i. *Aeromonas*

These organisms have been found to produce several toxins that may be important in pathogenicity. These include two cytotonic enterotoxins, one of which is cross-reactive with cholera toxin, and a cytotoxic enterotoxin which also cross-reacts with cholera toxin. The role of any of these toxins in diarrhea production is yet to be determined.

A subset of *Aeromonas* strains are invasive in the RITARD rabbit model, producing bacteremia, inflammatory diarrhea and death in the animals. Most of these strains are characterized by being agglutinable after boiling in culture media. Strains of *Aeromonas* isolated from patients with dysentery symptoms were significantly more often autoagglutinable than strains isolated from patients with watery diarrhea, suggesting that this simple marker may be useful in characterizing these strains.

j. *Enterotoxigenic Bacteroides fragilis*

Some strains of this species have been found to produce a heat labile cytotoxic enterotoxin, which can be assayed in animal model systems. Enterotoxigenic strains cause secretory responses in lamb ileal loops, gnotobiotic piglets, infant rabbits, and in the RITARD model. The pathologic lesions of inflammation and exfoliation of enterocytes are confined to the large bowel and distal ileum; there is no evidence of bacterial invasion.

The enterotoxin has not yet been purified or characterized in any detail. ETBF are recognized by picking typical colonies of *B. fragilis* from selective media, and assaying in a biological system.

In a study among Apaches in Arizona, children above the age of 1 year were found to have a significantly higher rate of

ETBF isolation than were control children without diarrhea. The rates of isolation were relatively high (19.7% vs 8.6%) and suggest that this organism could be an important newly recognized cause of diarrheal disease. The organism has also been identified in Bangladesh and in Peru, and prospective studies are underway to further characterize its potential importance.

7. Epidemiologic Studies of Diarrheal Pathogens:

Several community-based epidemiologic studies of acute diarrheal disease have been done. Studies among the Apaches in Whiteriver, Arizona, have shown 1) a high rate of diarrheal disease in children during the first year of life (~4/child/year) 2) a strong correlation between estimates of sanitation in the home and the diarrhea rates of children living in the home, and 3) a strong correlation between attendance at a large gathering, where sanitation is less than optimal, and subsequent diarrheal rates during the following week. A large array of diarrheagenic organisms were found, including ETEC, EPEC, Shigella, and rotavirus.

Epidemiologic studies of gastrointestinal disease among homosexual men in Seattle have been done. Both symptomatic and asymptomatic infection with identifiable enteric pathogens was found to be common, and multiple agents were frequently involved.

Epidemiologic studies of diarrheal disease among United States, and Mexican students studying in Mexico, and among Mexican children have been done. Major pathogens have been ETEC and enteroadherent strains of *E. coli*. The majority of students with shigellosis had rises in secretory IgA antibodies in stool. Studies in children suggested that enteroadherent strains of EPEC were important causes of diarrhea in that population.

Field areas will need to be developed for eventual large-scale efficacy studies of candidate diarrheal vaccines.

SIGNIFICANT ACCOMPLISHMENTS

The scientific accomplishments in the field of cholera and related diarrheal diseases research during the past 25 years are too numerous to give in any detail. They cover nearly the entire area of bacterial diarrheal disease research, since much of this research has been done with funding through the NIH. Some of the major areas of research will be briefly summarized, according to the five major goals of the panels, in their order of priority.

Development of effective vaccines for the control of cholera

Vaccine development has gone through a number of phases during this time. The first vaccines adequately field tested in Bangladesh (then East Pakistan) consisted of preparations of purified somatic antigens given parenterally. These vaccines gave protection for only about 6 months; subsequently, interest shifted to the development of a cholera toxoid vaccine. In 1974, studies in volunteers challenged with virulent *V. cholerae* showed that solid long-lasting protection could be induced by a clinical episode of cholera. Furthermore, studies in animal models were beginning to delineate the importance of the intestinal secretory immune system in protection against cholera, and studies began to focus on the mechanics of stimulating this secretory immune system optimally, and to determine which antigens could best stimulate protective immunity. It was generally agreed that oral immunization was the optimal route for stimulating protective immunity. Which antigens were most protective was not known, but it was decided to field test two nonliving oral cholera vaccines, one of which contained all the known cholera antigens from a culture of killed vibrios, and the other one that also contained the B subunit (the immunogenic, but physiologically inactive portion of the cholera toxin molecule-. This trial was carried out in the mid-1980's in Bangladesh through the International Center for Diarrheal Diseases Research, Bangladesh (ICDDRB), formerly the Cholera Research Laboratory. The study showed both vaccines, given in three doses, to provide significant protection over at least a 3-year period, and to be completely free of side effects. The vaccine containing the B subunit of cholera toxin provided superior protection, but only during the first 8 months after immunization; protection thereafter was equal. Older children and adults were better protected than younger children. The vaccine, though potentially a useful public health tool, is not currently being used.

The development of a candidate live oral vaccine has continued during the past 10 years, with the possibility that a single dose might provide significant long-lasting protection and without side effects. A number of candidate vaccines have been tested in volunteers in the United States. These studies have shown that colonization of the small bowel is the most important criterion for vaccine effectiveness. One of the major problems in these studies was developing a candidate vaccine that would not itself produce diarrhea in the volunteers. Such a vaccine is now available, and is currently being tested in volunteers in Thailand, a cholera endemic area.

Studies are continuing, however, in identifying the most appropriate antigens to include in the ideal live cholera vaccine, and how most optimally these may be given. Antigens which are clearly important besides the LPS and cholera toxin antigens, are the newly described TCP pilus antigens, outer membrane proteins, flagellar antigens, and cell-associated hemagglutinins. Most of these antigens are directed at producing an immune response which interferes with the colonization of the small bowel by *V. cholerae*. There is optimism about being able to develop such candidate vaccines which may be of public health importance.

As a background to this practical goal of vaccine development, much work was being done on dissecting the pathogenesis of *V. cholerae* infections. As a result, the pathogenesis of *V. cholerae* infection is perhaps one of the best known of all infectious diseases of man. The role of the cholera toxin in the production of the secretory response of the small intestine, and the subsequent clinical symptomatology was established. The cholera toxin was purified, its subunit structure characterized, the biochemical mechanisms for inducing secretion in the small bowel through cyclic nucleotide production determined, and its genetic control in the chromosome defined. The amino acid structure of the toxin molecule was identified, and parts of the molecule have been synthesized. In the course of this work, it became clear that there existed a family of related heat-labile [illegible]totonic enterotoxins that shared functional and antigenic determinants made by widely disparate organisms. These include not only enterotoxigenic *E. coli*, but strains of *V. cholerae* non-01, *Salmonella typhimurium*, *Aeromonas hydrophila*, and *Campylobacter jejuni*. With the exception of *E. coli* and some non-01 strains of *V. cholerae*, the importance of these enterotoxins in the pathogenesis of the diarrheal diseases produced, has not been determined.

Although for many years it was thought that *V. cholerae* did not produce pili, it has been recently shown that it produces at least three types of pili. One of them, the TCP pilus, is crucial for the vibrios to be able to colonize the small intestine and induce diarrheal disease, and appears to be an important protective antigen.

The therapy of cholera

Twenty-five years ago, cholera therapy depended upon the availability of intravenous fluids. The physiologic derangements had been well characterized, and the appropriate replacement solutions were well known. The lack of availability, cost, and difficulty of administration made it impossible to make this therapy available to most patients who needed it. Therefore, alternate methods of therapy were investigated, and it was found that fluids and electrolytes could be effectively replaced orally with solutions containing glucose and sodium in an equal molar ratio. It was also found that these solutions were effective, not only in cholera, but in all the dehydrating watery diarrheas. Thus, an oral replacement solution that was devised specifically for cholera has become a universal diarrhea treatment solution, and today is the mainstay of all diarrheal disease control programs around the world.

Efforts have continued to look for alternative substrates that may improve the efficiency of absorption, and thus decrease stool losses. This "therapeutic" effect has been achieved with cereal-based solutions containing rice, and with early feeding of lactose-free foods. Simplified home solutions of food such as rice gruel and wheat noodle soups have been adapted for use by the mother to treat children at home with diarrhea.

These three photos sequentially depict the dramatic effects of oral rehydration in a girl who was extremely dehydrated when she arrived at the hospital, but appeared normal 48 hours after receiving oral rehydration solution, which was administered by her mother.

The standard therapy for cholera now is 1) oral rehydration solutions, that are cheap, and can be administered by nonprofessional personnel, including the mother, 2) early feeding with calorie dense food, to combat the negative nutritional effects of the illness, and to augment the absorption of the oral rehydration solutions, and 3) antibiotics, such as tetracyclines to shorten the course of disease to about 36 hours. Tetracyclines have remained the drugs of choice, since they were first shown in the early 1960's to be effective.

Entertoxigenic *E. coli* (ETEC)

These organisms, first found to cause severe cholera-like diarrhea in Calcutta in the late 1960's, have a mechanism of pathogenesis almost entirely analogous to that of *V. cholerae*. Based on work that had been done with the cholera organisms, using similar animal models and other assay systems, the enterotoxins of these *E. coli* were identified. One of them, the heat-labile (LT) enterotoxin has been found to be closely related structurally and antigenically to cholera toxin, while the other, a heat-stable (ST) enterotoxin has been found to be quite different, consisting of a short polypeptide chain. Both toxins are controlled by genetic material found on plasmids, rather than on the chromosome, as is the case in *V. cholerae*. The genetic material, as well as the toxins, has been completely identified. The ST of *E. coli* has become the prototype for a larger family of ST's, all of which share similar structure and function. These ST's have now been identified in *V. cholerae* non-01, *Yersinia enterocolitica*, and in animal strains of *E. coli*.

Colonization factors called pili were first described in ETEC as important virulence factors and necessary in some strains for colonization and production of diarrheal disease; these are also controlled by plasmid genes.

ETEC are now known to be the most common bacteria causing acute diarrheal disease in most of the developing world's children, and are also the most common cause of the diarrhea experienced by travelers to the developing world. They are relatively infrequently found where sanitation levels are considered adequate.

Because of the diversity of antigens these strains carry, the development of vaccines has lagged considerably behind that of cholera. There are known at least 15–20 common O serotypes of ETEC, and at least 9 colonization factor antigens, in addition to the single antigenic LT and the nonantigenic ST. With the exception of ST, all of these antigens are potentially important in developing candidate vaccine strains. During the cholera vaccine field trial in Bangladesh, the cholera B subunit vaccine provided significant short-term protection against diarrhea caused by LT-producing ETEC.

Environmental and Ecological Studies

Although all the bacterial diarrheal pathogens have been well known to be transmitted by food and water, details of this transmission at the level of the home, and the survival of these organisms in the environment were not known. Studies over the past 15 years have established that *V. cholerae* 01 survive for prolonged periods of time in brackish waters, such as estuaries, especially associated with copepods, aquatic plants, and shellfish. Such environmental reservoirs are now known to serve as a source of human infections over prolonged periods of time, as has occurred along the U.S. Gulf of Mexico, where a single *V. cholerae* strain has caused intermittent outbreaks of cholera for at least the past 16 years. Nontoxigenic *V. cholerae* 01 are also found in brackish waters throughout the world, in both cholera endemic and nonendemic areas.

In the last 5 years, methods have been developed to facilitate the study of these organisms in the environment. *V. cholerae* organisms which are viable, but nonculturable have been found in the environment. Such strains can be detected by immunologic methods as well as methods which detect the DNA of the organisms. These studies indicate that cholera vibrios are much more widespread in the environment than previously recognized.

Other bacterial diarrheal pathogens

The prototype invasive diarrheal pathogen is *Shigella*. The large molecular weight plasmid carried by all strains of Shigella and invasive *E. coli* has been of particular interest recently because of the invasion protein antigens which are encoded there. These antigens are common to all of the different strains carrying them, and therefore might be useful both in developing diagnostic assays and for consideration for use in vaccine strains.

The Shiga toxin, produced by *S. dysenteriae* type 1, is of interest because of its relation to the Shiga-like toxins of *E. coli*, discussed later, and because of the possibility that it is involved in the dysenteric disease produced by this organism. The toxin has been purified, and its receptors identified. Although it can be identified in the stools of patients infected with *S. dysenteriae*, its role in the disease process is not clear.

The diversity of mechanisms by which *E. coli* cause diarrhea expanded to include not only strains which produced enterotoxins, like *V. cholerae*, or were invasive, like *Shigella*, but also strains which were found to adhere to and cause effacement of enterocyte brush border membranes (designated enteroadherent) and strains which produced Shiga-like toxins (designated enterohemorrhagic).

Enteroadherent strains of *E. coli*, defined by their characteristic adherence to tissue culture cells, could be defined into three patterns: localized, diffuse, and autoaggregative. The genetic control of these adherence phenomena is located both in plasmid and chromosomal DNA, and probes have been developed to allow for their ready identification. Both the localized and autoaggregation strains are epidemiologically related to acute diarrhea in children in developing countries. Many enteropathogenic serotypes are also enteroadherent, and the classification of these organisms has yet to be fully worked out.

An entirely new group of *E. coli*, the enterohemorrhagic strains (EHEC) has been described in the past several years which produces Shiga-like toxins. EHEC are etiologic in both a syndrome of hemorrhagic colitis and in the Hemolytic Uremic Syndrome (HUS). They produce at least three known Shiga-like toxins (or Vero toxins), known to be controlled by toxin-converting bacteriophages. These toxins have all been purified and characterized, and the genetic material which controls them cloned and sequenced. Their mechanism of action is through the inhibition of protein synthesis in a manner analogous to that of Ricin, a plant toxin. The SLT's do seem to be directly involved in the pathogenesis of the HUS since these toxins bind to receptors on vascular endothelium and kidneys, and result in toxic damage to those cells. The Shiga toxin and SLT I are identical but for a single amino acid difference. These toxins define yet a third family of toxins produced by diarrheagenic bacteria. These organisms have been involved mostly in outbreaks of disease traced to contaminated processed food. They seem to be infrequently found in the developing world, where food processing technology is not in use.

A number of new bacteria have been found to produce cholera-like enterotoxins, such as *Salmonella*, *Aeromonas*, and *Camplyobacter*, but the role of this toxin in the disease is unclear. Some bacteria, like *Y. enterocolitica*, produce an ST, similar to that of *E. coli*, but without evidence that the toxin is involved in the disease. Other organisms that produced enterotoxins, such as *Clostridium difficile*, *Clostridium perfringens*, *Staphylococcus aureus* have been extensively studied, and their enterotoxins purified and characterized. Some well-known organisms, like *Bacteroides fragilis*, have been recently found to produce enterotoxins, which have not yet been characterized. Some strains, such as *Aeromonas*, produce several cytotonic and cytotoxic enterotoxins, as well as being invasive, but the role of any of these characteristics in the disease process is not known.

PROJECTS SUPPORTED BY THE PROGRAM

GRANTS

Investigator and Institution	*Title*
Joan Butterton Massachusetts General Hospital	Iron-regulated virulence factors in *Vibrio cholerae*
Stephen B. Calderwood Massachusetts General Hospital	Genetic and biochemical analyses of Shiga-like toxins
Jorge Crosa Oregon Health Sciences University	Iron uptake as a virulence factor in pathogenic vibrios
Arthur Donahue-Rolfe New England Medical Center	*Shigella* toxin: Structure and function relationships
Herbert DuPont University of Texas, Houston	U.S./Mexican students' susceptibility to enteric pathogens
Richard A. Finkelstein University of Missouri	*Escherichia coli* LT-related enterotoxins
Richard A. Finkelstein University of Missouri	Cholera: Pathogenesis and immunology
Jean Gariepy Cancer Institute	Characterization of the *Escherichia coli* Ontario enterotoxin ST1 receptor
Michael Gill Tufts University	Mechanisms of action of cholera toxin
James B. Kaper University of Maryland	Molecular genetics of enteropathogenic *Escherichia coli* adhesion
Frederick T. Koster University of New Mexico	Antigen handling in mucosal immunity to toxins and pili
Gerald Keusch New England Medical Center	*Shigella* exotoxin: Cell receptors and mode of action
Anthony Maurelli Henry Jackson Foundation	Temperature regulation of *Shigella* virulence genes
Steve Moseley University of Washington	New adhesins of diarrhea-associated *Escherichia coli*

Alison D. O'Brien Uniformed Services University	Shiga-like toxin of *Escherichia coli*
Tom G. Obrig Albany Medical College	Shiga toxin mode of action in bacterial disease
Sunil Palchaudhuri Wayne State University	Mechanisms of *Shigella dysenteriae* adhesion and internalization
Kenneth Peterson Louisiana State University	Analysis of *Vibrio cholerae* genes involved in colonization
Kathleen Richardson Oregon Health Sciences University	Role of flagella in *Vibrio cholerae* pathogenesis
Donald C. Robertson University of Kansas	Pathogenicity of enterotoxigenic *Escherichia coli*
June Scott Emory University	Regulation of expression of adherence factors
Pamela Small Middlebury College	Invasion determinants in enteroinvasive *Escherichia coli*
Ronald K. Taylor University of Tennessee	Genetic Determinants of virulence in *Vibrio cholerae*
Arnold White University of Missouri	Activation of intestinal mucosa guanylate cyclase
Thomas S. Whittam Pennsylvania State University	Clonal nature of cytotoxigenic *E. coli*

CONTRACTS

Myron M. Levine University of Maryland	A facility for the study of infectious diseases, vaccines, and other antimicrobial agents in community volunteers

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Japanese Cholera and Related Diarrheal Diseases Panel

BROAD GOALS

The Japanese Cholera and Related Diarrheal Diseases Panel has carried on joint research with the U.S. Panel on the following: (1) pathogenic factors produced by *Vibrio cholerae* and related bacteria; (2) bacteriology of *Vibrio cholerae* and related bacteria; (3) diarrheal diseases due to cholerae-like enterotoxins; and (4) immunity of cholera and related vibrios. The ultimate objective of this joint research is to decrease the morbidity and mortality of cholera and related diarrheal diseases. For this purpose, studies have been performed in bacteriology and genetics, on structure and function of toxin and other virulence factors, in pathology, and in immunology of toxins and various bacterial cellular antigens.

The most important objective of the joint research is to develop an immunogen with immunizing capacity superior to the protection afforded by conventionally killed vaccines. To achieve this objective, rigorous studies are underway by many investigators.

The occurrence of diarrheal diseases in humans cannot be optimally effective unless research on not only *V. cholerae*, but also other vibrios such as *V. cholerae* non-01 and *V. parahaemolyticus*, enterotoxigenic *E. coli*, *Shigella*, and others are carried out. Studies are now in progress on the virulence factors and on the pathophysiology of these bacteria.

PROGRESS IN PRIORITY AREAS

Bacteriology of *V. cholerae* and related diarrheagenic bacteria

Chemical structure of cell wall lipopolysaccharide (LPS) of *V. cholerae* 01 and *V. cholerae* bio-serogroup HAKATA has been well characterized. A 2-Keto-3-deoxy-octonate (KDO)-like substance of LPS of *V. parahaemolyticus* has been identified as 2-Keto-3-deoxy-threo-hexonic acid.

To characterize the antigenicity of *V. cholerae*, several monoclonal antibodies against *V. cholerae* 01 O-antigens are prepared. They are subdivided into three groups: the first group has the identical reactivity to both Ogawa and Inaba serotypes, the second reacts with only Inaba serotype, and the third is reactive mainly with Inaba serotype but weakly with Ogawa serotype.

Genetic studies on *V. cholerae* and related diarrheagenic bacteria

Genetic studies on the genes involved in epithelial invasion of *Shigella flexneri* and *S. sonnei* have showed great progress. In *S. flexneri* and *S. sonneri*, 7 and 8 genes, respectively, are demonstrated to be involved in invasion of the intestine. The nucleotide sequences of some of the genes of the virulence-associated region in the chromosome have been determined.

The genetic mechanism of the production of heat-labile enterotoxin (LT) by enterotoxigenic *Escherichia coli* has also been studied. Strains which produce significantly different amounts of LT carry almost identical amount of mRNA for LT. Thus, it is concluded that expression of LT gene is not regulated at the transcriptional level. The downstream region of the LT gene is assumed to play a role in regulation of the expression of the LT operon.

In *V. parahaemolyticus*, evidence has been found that the gene for the thermostable direct hemolysin moves as a transposition between different replicons.

Studies on toxins and other virulence factors produced by *V. cholerae* and related bacteria

Cholera toxin activates the membrane-bound adenylate cyclase of the target cells. Extensive work on the biochemical mechanism of the activation of adenylate cyclase has been carried out, and the mechanism of interaction between ADP-ribosylation factors needed for activation of cholera toxin and the A1 fragment of cholera toxin has been studied.

The structure and function relationship of heat-stable enterotoxin (ST) of enterotoxigenic *E. coli* has been well characterized. The positions of three disulfide linkages in ST are determined and the importance of these linkages in ST-activity is demonstrated. Synthetic ST-analogues with single replacements of amino acid residues with D-amino acid have been prepared and biological and physicochemical properties of these analogues have been studied. The ST-analogue with D-Cys at position 5 is a strong agonist, suggesting that the analogue is a possible candidate for a nontoxic, immunogenic toxoid.

A newly recognized diarrheagenic bacteria, enterohemorrhagic *E. coli*, produces two distinct toxins that kill Vero cells (Vero toxin, VT). One of them, VT1 or Shiga-like toxin I, is found to be identical in the amino acid sequence as well as the nucleotide sequence of the gene to Shiga toxin produced by *Shigella dysenteriae*. The other, VT2 or Shiga-like toxin II, has about 60% homology in the amino acid sequence and the nucleotide sequence to Shiga toxin.

Studies to elucidate the mode of action of VT1 and VT2 have been carried out. Both toxins as well as Shiga toxin show RNA N-glycosidase activity and cleave the N-glycosidase bond of an adenosine at position 4324 from the 5′ terminus of 28S ribosomal RNA of 60S ribosomal subunit of eukaryotic cells. This enzymatic activity results in inhibition of protein synthesis in eukaryotic cells. How this biochemical reaction relates to the symptoms of enterohemorrhagic *E. coli* and *S. dysenteriae* remained to be determined.

Thermostable direct hemolysin (TDH) of *V. parahaemolyticus* is well recognized as an important virulence factor. TDH-like hemolysin (TLH) has been isolated and purified, and its role as a virulence factor has been studied. The nucleotide sequence of the gene for TLH has been determined.

Studies on infection and its mechanism of *V. cholerae* and related bacteria

The inhibitory effect of various kinds of calcium antagonists in cholera toxin-induced secretory diarrhea has been studied. It has been concluded that Ca^{++}-mediated pathway participates in cholera toxin-induced intestinal secretion.

The mechanism of suppression of T-cell responses by cholera toxin is also studied. Cholera toxin causes a relative increase of L3T4-cells and a decrease in L3T4+ T cells. In further study based on the above observations, it has also been demonstrated that an organ-specific autoimmune phenomenon, insulitis, can be prevented and the resultant diabetes treated by cholera toxin in the experimental animal.

The adherence of *V. cholerae* 01, *V. cholerae* non-01, *Vibrio parahaemolyticus* and enterotoxigenic *Escherichia coli* (ETEC) to human small intestinal mucosa have been studied with samples excised from patients with ascending colon cancer. *V. cholerae* 01 and *V. cholerae* non-01 adhere, in decreasing order, to mucus coating, the epithelial surface (especially M cell) of ileal lymphoid follicles, and the epithelial surface of ileal or jejunal villi; *V. parahaemolyticus* adheres, in decreasing order, to the epithelial surface (especially M cell) of ileal lymphoid follicles, the epithelial surface of villi, and the mucus coating. ETEC adhere more to the epithelial surface of ileal lymphoid follicles than to epithelial surface.

SIGNIFICANT ACCOMPLISHMENTS

Cholera Toxin: Its Two Antigenic Components (Subunits) and Three-Dimensional Molecular Shapes.

In the "First Conference on the Cholera Problem" held from July 26 to 29, 1988 at the Imperial Department of Health in Berlin, Robert Koch mentioned in his discussion that "Ich Mochte nur betonen dass sich im Darminhalt unter dem Einfluss der Kommabazillen eine toxische Substanz dildet." The toxic substance, initially designated choleragen and now called cholera toxin or cholera enterotoxin, was purified to homogeneity in 1969 from the culture supernatant of *Vibrio cholerae* 01, a causative agent of cholera. However, there were problems left to be solved for further critical understanding about its molecular nature. Investigations in 1969 found choleragenoid at the last step of purification of cholera toxin. Choleragenoid was immunologically identical with cholera toxin but was lacking in toxicity for model animals, and thus it was the spontaneously formed toxoid.

In 1972, large-scale methods using 200-liter pilot fermentors were developed for producing purified cholera toxin. The methods yielded ca. 1 mg of purified cholera toxin from 1 liter of culture of *V. cholerae* 01 (strain 569B; classical biotype, Inaba serotype). Horse and rabbit antisera to the purified cholera toxin were then prepared in a large scale. Using those substances, investigators in 1972 clearly demonstrated that cholera toxin and choleragenoid can immunologically be distinguished from each other—the data were presented at the 9th U.S.-Japan Joint Cholera Conference held at Grand Canyon, Arizona, in October 1973. When cholera toxin and choleragenoid were analyzed by the agar gel immunodiffusion tests, horse antiserum to cholera toxin gave a definite precipitin line (against both cholera toxin and choleragenoid) with a spur extending the cholera toxin (choleragen)-precipitin line beyond the choleragenoid-precipitin line. The result unambiguously indicated the presence of antigenic determinants not only common between cholera toxin and choleragenoid but also unique to cholera toxin. The spur-bearing antibody was then isolated by means of affinity chromatography. This spur-bearing antibody reacted with cholera toxin but not with choleragenoid, as expected. Based on the observations, scientists concluded in 1973 that (i) cholera toxin consisted of two antigenic components (subunits): one being essential for the production of toxicity and one being nontoxic; and (ii) choleragenoid lacked the former component.

Around the same time or immediately afterward, the subunit structure of cholera toxin was reported in detail by investigators in Sweden and the United Kingdom; their data confirmed the work of Japanese and American scientists. There is now a consensus that the cholera toxin molecule consists of one subunit A moiety and five subunit B moieties. The subunit A undergoes post-translational processing to generate A1 and A2 peptides; A1 and A2 are linked to each other by a single disulfide bond, and A2 is presumably important for binding of A1 to the subunit B. The subunit B binds to the receptor (GM1 ganglioside) of the target cells. Subsequently, the subunit A (A1-A2) penetrates across the membrane and the A1 portion (ADPT-ribosyltransferase), separated from the rest of the molecule, catalyzes the ADP-ribosylation of Gs alpha, the stimulatory GTP-binding protein of the adenylate cyclase system, resulting in elevation of intracellular cyclic AMP levels. In contrast, choleragenoid consisted of oligomers of the subunit B. Thus, the spur-bearing antibody, reported in 1974, was an antibody that specifically recognizes the subunit A in cholera holotoxin. This fact also indicated that some epitopes of the subunit A are located on the outside surface of the cholera toxin molecule.

The three-dimensional molecular structures of purified cholera toxin and choleragenoid were analyzed in the electron microscope by several collaborating investigators in 1975 and 1976—the data were presented at the 10th U.S.-Japan Joint Cholera Conference held in Kyoto, October 1974. In order to visualize the cholera toxin or choleragenoid molecules, the negative-contrast technique was used in those studies. The specimens were analyzed in a transmission electron microscope with an accelerating voltage of 75-80 kV and direct magnification of 50,000–80,000X. Cholera toxin molecules observed were almost uniform in size and had a unique three-dimensional structure. In this case, the subunit B molecules were arranged in a ring form or like flower petals around a center core of the subunit A. The number of the subunit B molecules consisting of a ring form was estimated to be five or six. The topological relation of each protein element varied under the test conditions—it was considered that those elements may not be rigidly combined in the toxin molecule. In contrast, in the case of choleragenoid, only a ring form of the subunit B was observed, and a center core which was detected with native cholera toxin was lacking. The scientists thus concluded with respect to the three-dimensional shape of cholera toxin that the subunit A molecule lies at the center and five or six subunit B molecules construct a ring-form structure surrounding the center core.

The subunit arrangement (number) of cholera toxin, which was determined by the electron microscopy, was in excellent agreement with the subunit structure as determined by gel electrophoresis or other methods.

PROJECTS SUPPORTED BY THE PROGRAM

Investigator and Institution	*Title*
Dr. Yoshifumi Takeda Professor Faculty of Medicine Kyoto University Yoshida-Konoe-cho Sakyo-ku, Kyoto, Japan	1) Structure and function of Vero toxin (Shiga-like toxin) produced by faculty of enterohemorrhagic *Escherichia coli* 2) Analysis of the thermostable direct hemolysin (TDH) and its related genes of *Vibrio* species
Dr. Kazunobu Amako Professor Department of Bacteriology Faculty of Medicine Kyushu University 3-1-1 Maedashi, Higashi-ku Fukuoka, Japan	Molecular study on the survival mechanism of *Vibrio cholerae* 01 in environment
Dr. Masaaki Iwanaga Professor Department of Bacteriology Faculty of Medicine 207 Uenohara, Nishihara-machi Nakatoh-gun, Okinawa, Japan	1) Purification and characterization of pili produced by *Vibrio* and *Aeromonas* species 2) Adherence of *V. cholerae* 01 and *A. hydrophila* to rabbit small intestines
Dr. Yasuo Kudoh Chief Department of Microbiology Tokyo Metropolitan Research Laboratory of Public Health 3-24-1 Hyakunin-cho Shinjuku-ku, Tokyo, Japan	1) Enteropathogenicity of *Aeromonas* species clinical isolates 2) Verocytotoxin study of *E. coli* serotype 0145 isolated from outbreaks in Tokyo
Dr. Tatsuo Yamamoto Assistant Professor Department of Bacteriology School of Medicine Juntendo University 2-1-1 Hongo, Bunkyo-ku Tokyo, Japan	1) Adherence of enteropathogenic bacteria to human intestines and M cells 2) Evolutionary origin of cholera toxin and *E. coli* heat-labile enterotoxin
Dr. Hitoshi Asakura Professor The 3rd Department of Internal Medicine School of Medicine Niigata University 757 Ichiban-cho Asahichodori Niigata, Japan	1) Haemorrhagic enterocolitis induced by *Clostridium difficile* toxin 2) Mechanism on diarrhea induced by cholera toxin
Dr. Nobuya Ohtomo Director Department of Microbiology Oita Prefectural Institute of Health and Environment Hoogawara-danchi, Oita, Japan	Enteropathogenicity of *V. parahaemolyticus*

Dr. Makoto Ohashi Director-General Tokyo Metropolitan Research Laboratory of Public Health 3-24-1 Hyakunin-cho Shinjuku-ku, Tokyo, Japan	Epidemiology and identification of gastrointestinal etiologic agents
Dr. Noboru Okamura Chief Department of Microbiology The Institute of Public Health Minato-ku, Tokyo, Japan	Immunological study on antibody responses in shigellosis
Dr. Sachio Ogata Professor Department of Microbiology Kyorin University School of Medicine Mitaka, Tokyo, Japan	1) Toxigenesis study on *V. cholerae* 01 in mice 2) Genetics of bacterial heat shock proteins
Dr. Akira Ghoda Director DI Center, Kitasato Institute, Minato-ku Tokyo, Japan	Application of gray musk shrew (*Suncus murinus*) in research on diarrheal diseases
Dr. Tadakatsu Shimamura Professor Department of Bacteriology Showa University 1-5-8 Hatanodai Shinagawa-ku, Tokyo, Japan	1) Conditions of cholera toxin production by *V. cholerae* 01 2) Effects of tea on cholera
Dr. Shoichi Shimotori Professor School of Health Sciences Kyushu University 3-1-1 Maedashi, Higashi-ku Fukuoka, Japan	Immunohistochemical study of cholera toxin in rats
Dr. Yasutsugu Shimonishi Professor Institute for Protein Research Osaka University, Suita Osaka, Japan	Molecular structure and active sites of heat-stable enterotoxins produced by *E. coli* and other enteric bacteria
Dr. Tae Takeda Director Department of Infectious Diseases Research National Children's Medical Research Center Setagaya-ku, Tokyo, Japan	Study on heat-stable enterotoxins by using monoclonal antibodies and DNA sequences
Dr. Masaharu Tsuchiya Professor Department of Internal Medicine School of Medicine Keio University 35 Shinanomachi Shinjuku-ku, Tokyo, Japan	Intracellular mechanisms induced by bacterial toxins

Dr. Tatsuro Naito Professor Department of Bacteriology Institute for Tropical Medicine Nagasaki University, Nagasaki, Japan	Purification and characterization of pili produced by *V. cholerae* O1
Dr. Rintaro Nakaya Professor Department of Microbiology Tokyo Medical and Dental University School of Medicine 5-45 Yushima 1 chome, Bunkyo-ku, Tokyo, Japan	Plasmids and pathogenicity of *Salmonella braenderup* and *S. typhimurium*
Dr. Hideo Hayashi Professor Department of Microbiology Kagawa Medical School Miki-cho, Kagawa, Japan	Genetic study on heat-labile enterotoxin expression in *E. coli*
Dr. Kazuhito Hisatsune Professor Department of Microbiology School of Pharmaceutical Sciences Josai University Sakado, Saitama, Japan	Chemical study on O-antigenic lypopolysaccharides of *Vibrio* species
Dr. Takeshi Honda Associate Professor Research Institute for Microbial Diseases Osaka University 3-1 Yamadaoka Suita, Osaka, Japan	1) Characterization and molecular genetics of toxins produced by *Vibrio* species 2) Adhesins of enterotoxigenic *E. coli*
Dr. Toshio Miwatani Professor Research Institute for Microbial Diseases Osaka University 3-1 Yamadaoka Suita, Osaka, Japan	1) Purification and analysis of virulence factors of enteropathogenic bacteria 2) Genetics of toxins produced by *Vibrio* species
Dr. Sumio Yamada Department of Microbiology Tokyo Metropolitan Research Laboratory of Public Health Tokyo, Japan	Serological diagnosis of enteropathogenic bacteria
Dr. Takeshi Yokota Professor Department of Bacteriology and Medicine Juntendo University 2-1-1 Hongo, Bunkyo-ku Tokyo, Japan	1) Adherence of bacterial enteropathogens to human intestines and M cells 2) Genetics of bacterial toxins and O-antigens
Dr. Haruo Watanabe Director Department of Bacteriology N.I.H., Shinagawa-ku Tokyo, Japan	Molecular genetics and cell-invasion mechanisms of *Shigella* species

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Joint Scientific Conferences

CHOLERA AND RELATED DIARRHEAL DISEASES PANELS

Date: November 6–8, 1984

Place: Nara, Japan

Summary: The intense interest in the mucosal immune response to cholera immunization continued in this year's presentations. Animal studies continue to support the superior immunogenicity of live bacteria, regardless of whether they produce enterotoxin or toxin subunits. Furthermore, data presented at the meeting showed that oral immunization is less efficient in females during periods of lactation and in infants who are nursing on milk containing high levels of antibody.

Studies of cholera pathogenesis in mice have shown a genetic basis for resistance to cholera toxin-induced secretion and suggest that sensitivity is mediated in part by cells from the bone marrow. Studies of the pathogenesis of enterotoxigenic *E. coli* have focused on the heat-stable enterotoxins (ST), which have now been completely sequenced. All 18- and 19-amino acid toxins have virtually identical core sequences.

Genetic studies of *V. cholerae* have focused on the regulatory genes controlling enterotoxin production. In ETEC, the genes controlling STb production reside on a transposon, whereas the genes controlling Shiga-like toxin reside on specific bacteriophages. The genes are highly related to the cytotoxin genes on the chromosome of *Shigella* species. Some strains of *E. coli* have been found to produce very high quantities of these Shiga-like toxins.

Date: October 21–23, 1985

Place: Bethesda, Maryland

Summary: Vaccine development was a highlight of this meeting. New, recombinant strains of *V. cholerae* have been constructed, and preliminary volunteer studies have shown that these strains are non-diarrheagenic but highly protective. These strains may become candidates for field trials and would have the advantage of stimulating protection after a single oral immunization. In the meantime, results from a large-scale field trial in Bangladesh have shown that killed cholera vaccines (with or without B subunit) were significantly protective during the first 6 months of the trial. The whole cell vaccine gave 58% protection vs. 85% for the whole cell + B subunit vaccine.

Several studies of the role of iron-regulated proteins and cell-associated hemagglutinins in pathogenesis were presented at the meeting. In addition, studies of the genetic control of host response to cholera toxin have shown that this property is in part controlled by the major histocompatibility complex, although receptor interactions and different subpopulations of cells also contribute to this effect.

Other important presentations were given on the structure of Shiga and Shiga-like toxins and on the potential role of enteroadhesive *E. coli* in diarrhea in children. Two distinct receptors for the Shiga toxin B subunit have been described. The receptors are developmentally regulated and are not detectable in animals younger than 18 days, when they become sensitive to Shiga toxin.

Date: July 20–23, 1986

Place: Toyama, Japan

Summary: Vaccine studies of live, oral cholera vaccines continue to be promising. One vaccine developed at the University of Maryland (CVD 103) is highly immunogenic and protective, while producing few side effects. Other studies showed that the vaccine strain must colonize the intestine well in order to adequately stimulate the mucosal immune response and that a pilus colonization factor in *V. cholerae* is required for colonization and is coordinated regulated with cholera toxin production.

The rapid pace of research on enterotoxins was clearly shown in the presentations at this meeting. The Shiga toxin and Shiga-like toxins of *E. coli* and *V. cholere* have been further identified and characterized through nucleotide sequencing and cloning. The B subunit of Shiga toxin is only distantly related to the B subunit of *E. coli* LT and *V. cholerae* classical enterotoxins. The Shiga toxin also has been shown to affect only the villus cells and not the crypt cells of the intestine. The toxin seems to cause decreased absorption in villus cells without influencing the secretory activity of the crypt cells, a process similar to the way viruses invoke a diarrheal response. Other reports compared the nucleotide sequence similarities between Shiga toxin and the other Shiga-like toxins.

Date: November 10–12, 1987

Place: Williamsburg, Virginia

Summary: Data from the first year of followup in the oral killed *V. cholerae* field trial in Bangladesh were presented. Protection was 53% in the group who received the whole cell vaccine and 62% for those who received the whole cell + B subunit vaccine. Protection was greatest in persons over 6 years old. A derivative of the live, oral *V. cholerae* vaccine (CVD 103) has been constructed to contain a gene encoding mercury resistance. This strain is immunogenic and safe in volunteers and provides good heterologous protection.

Presentations from studies with enterotoxigenic *E. coli* (ETEC) vaccines included studies of an oral, nonliving vaccine that had been prepared by treating *E. coli* with colicin E2. This vaccine stimulated intestinal IgA responses in volunteers and protected against homologous challenge in 24 volunteers. A bovine colostrum concentrate obtained from cows immunized with ETEC was shown to be completely protective against challenge with ETEC in volunteers. These data suggest that this approach may be useful for prophylaxis in travelers diarrhea.

Several presentations also focused on genetic regulation of cholera toxin and pilus formation as well as the structure and activities of toxins produced by *Shigella*, *E. coli*, *Aeromonas*, and *Bacteroides*.

Date: November 13–15, 1988

Place: Tokyo, Japan

Summary: One of the major themes at this meeting was the genetic regulation of toxin and pili expression in *V. cholerae*. Several studies showed that many genes—most of which have not been characterized—are coordinately regulated by central regulatory gene(s) such as *ToxR*. There were also several studies describing the binding mechanisms, receptors, and structure of the enterotoxins of noncholera organisms, especially enterotoxigenic *E. coli* and *Shigella*.

The 3-year followup of the killed, oral *V. cholerae* vaccine trial in Bangladesh showed that the cumulative protective efficacy for the entire interval was 50% for the whole cell + B subunit and 52% for the whole cell vaccine alone. Efficacy was less for children vaccinated between 2 and 5 years of age (26% and 23%, respectively). Vaccine efficacy was 63% and 68% for persons over 5 years of age, and the vaccine effect was sustained in older persons. The live, oral vaccine developed at the University of Maryland was shown to be safe and immunogenic in volunteers in Thailand.

Other key presentations focused on the genes responsible for invasion by *Shigella*, the synthesis and processing of enterotoxins in *E. coli*, and the potential role of enterotoxigenic *Bacteroides fragilis* as a pathogen in older children and adults.

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Profiles

U.S. ENVIRONMENTAL MUTAGENESIS AND CARCINOGENESIS PANEL

Dr. I. Bernard Weinstein

Chairman (1987–Present)

I. Bernard Weinstein, M.D., is Director, Comprehensive Cancer Center and Frode Jensen Professor of Medicine, Columbia University. He received his M.D. degree from the University of Wisconsin, Madison, Wisconsin, in 1955, was a Clinical Associate at the National Cancer Institute, Bethesda, Maryland, and a Research Associate at the Massachusetts Institute of Technology, Boston, Massachusetts, prior to going to Columbia University in 1961. Dr. Weinstein is a world-renowned expert in the molecular mechanisms of carcinogenesis, particularly in the area of tumor promotion and oncogene activation. He has authored over 370 publications, is on the Editorial Board of 8 major publications, is a member of numerous professional societies, and is on the advisory boards of a number of international and interagency committees.

JAPANESE ENVIRONMENTAL MUTAGENESIS AND CARCINOGENESIS PANEL

Dr. Taijiro Matsushima

Chairman (1986–Present)

While he was working at the Panel Chairman's Institute, Dr. Matsushima contributed much to Panel activities by assisting the Chairman and organizing all of the Joint Panel Conferences since the 5th meeting in 1975. He became a panel member in 1978 and served as the third panel Chairman in 1986.

Dr. Matsushima graduated from Osaka University, Faculty of Science, where he trained in protein chemistry and enzymology. He worked on enzymology of cancer cells, chemical carcinogenesis, metabolism of carcinogens, and environmental mutagenesis.

Dr. Takeo Suzuki

Chairman (1981–1986)

Dr. Suzuki graduated from the Imperial University of Tokyo, Faculty of Medicine in 1940 and started his 45 years of research work at the National Institute of Public Health. He devoted himself to the studies on occupational and environmental health and air pollution control, and also put his heart into the public health education until his retirement as the Director of that Institute in 1985.

Dr. Suzuki is a broadminded person with a gentle personality and persuasive power, who showed foresight and enthusiasm during his leadership in research on environomental health. He was a charter member of the Environmental Mutagenesis and Carcinogenesis Panel and served as Panel Chairman from 1981 to 1986. Much of the achievement and success of this Panel was due to his efforts.

He served as the chairman or member of many national committees of Ministry of Health and Welfare and the Agency for Environment and contributed a great deal to policies of public health and environmental problems.

Dr. Suzuki was president of the Air Pollution Control Association from 1971 to 1987, and he also served internationally as a member of WHO Expert Committee on Environmental Health and UNEP Expert Committee on World Environment Report.

Dr. Tadashi Yamamoto

Chairman (1972–1981)

Dr. Yamamoto loved experiments in the laboratory and started his research career as a medical student at the Institute of Infectious Disease, University of Tokyo, which changed later its name to the Institute of Medical Science. He continued his research in this Institute until his retirement in 1978. Dr. Yamamoto served as the Director of the Institute from 1973 to 1977. He studied microbiology, immunochemistry, and cell biology but later he changed to cancer research after his brother died of cancer. He worked on cancer chemotherapy and RNA tumor virus. He also served as medical doctor in the Navy during World War II.

Dr. Yamamoto always showed interest in others and was a good organizer and coordinator in bringing together scientists in fields different from his own where he had broad knowledge and genuine foresight on both fundamental and clinical medical issues. When the Environmental Mutagenesis and Carcinogenesis Panel was established, he was asked to serve as its first chairman, and he encouraged and stimulated young scientists to work in the area of environmental mutagenesis and carcinogenesis. With his leadership, the Panel progressed with great success.

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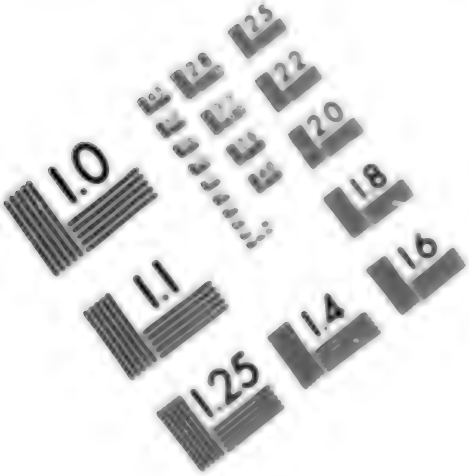

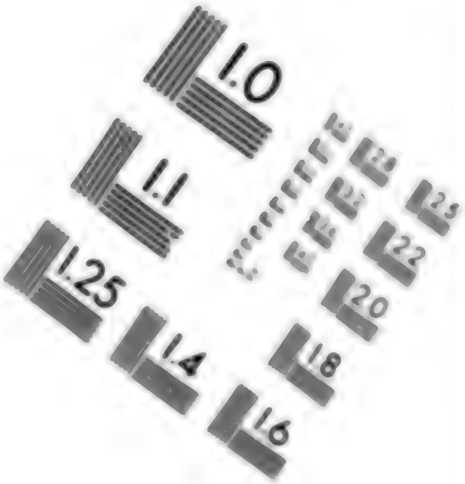

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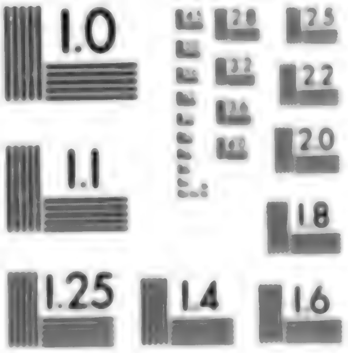

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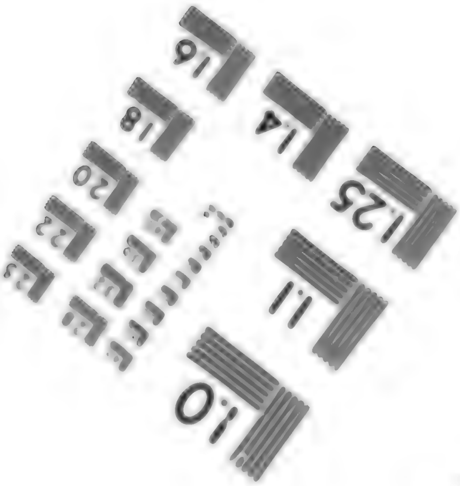

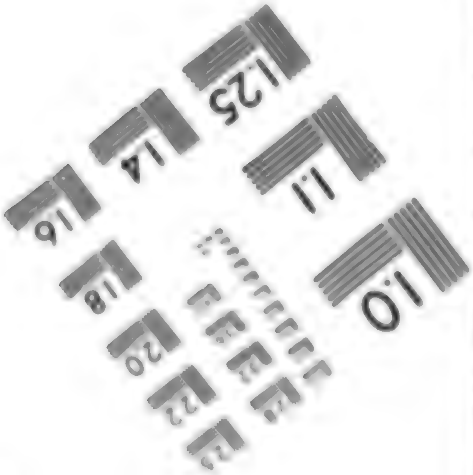

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GUIDELINES

ENVIRONMENTAL MUTAGENESIS AND CARCINOGENESIS PANELS
United States-Japan Cooperative Medical Science Program

There is expanding evidence that chronic human diseases, including cancer, can be caused by man-made and natural chemicals as well as other agents in the human environment. This program focuses on two aspects of such environmental disease; mutagenicity and carcinogenicity. Approaches to these can be broadly divided into laboratory and epidemiologic studies. For both, one goal is to apply and transfer knowledge to world populations, particularly in Asia, developing countries, and high-risk areas.

A. Laboratory approaches to the detection, prediction, interpretation, and mechanisms of the mutagenic and carcinogenic properties of chemical and physical agents.
   1. Development, refinement, validation, and application of laboratory assay systems for assessing mutagenicity and carcinogenicity.
   2. Systemic collection and evaluation of data base on mutagens and carcinogens.
   3. Studies of the relationships:
      a. between mutagenicity and carcinogenicity
      b. in the toxic responses of various species, especially microbial, rodent, and human
      c. between somatic and germ cell mutagenicity
      d. among types of genetic lesions, e.g., gene mutations and chromosomal aberrations
      e. between initiation and promotion in carcinogenesis
      f. between chemical structure and toxicity
      g. between pharmacokinetics/metabolism and toxicity
   4. Investigation of factors which modify mutagenic and carcinogenic activity, including dose-time effects, repair processes, and multiagent interactions.

B. Epidemiologic approaches to understanding and preventing environmental mutagenesis and carcinogenesis in human populations.
   1. Development, refinement, and validation of methods to determine exposure, DNA dosimetry, and somatic and germ cell mutations' early stages of carcinogenesis in human populations.
   2. Application of human monitoring methods to determine baseline frequencies of mutation or other monitored endpoints and to monitor high-risk groups such as chemotherapy patients, workers exposed to known or suspected mutagens and carcinogens, and individuals or families genetically predisposed to genetic damage and cancer.
   3. Promote interdisciplinary studies of human mutagens and carcinogens.
   4. Development of qualitative and quantitative methods for monitoring the workplace and the general environment (air, water, soil, food) for the presence and levels of mutagenic or carcinogenic agents.
   5. Furthermore, epidemiologic methodology and expansion of epidemiologic resources to measure mutagenicity and carcinogenicity by agents in the human environment.
   6. Combining of laboratory studies of agents, human hazards, and human exposure into a sound, scientifically based risk analysis for mutagenicity and carcinogenicity.

U.S. Environmental Mutagenesis and Carcinogenesis Panel

PROGRESS IN PRIORITY AREAS

During the past several years, the Joint Panel has focused on several newly emerging areas of research, reflecting the increasing influence that basic concepts and research technologies in molecular biology are having on the fields of environmental mutagenesis and carcinogenesis. For example, research has focused on understanding the role that oncogenes play in the process of tumorigenesis at the molecular level in both experimental animal tumors and human tumors. Recent evidence suggests that a small family of normal cellular genes, called protooncogenes, can undergo mutation to form cancer-causing oncogenes. Characterization of oncogene activation in human and rodent tumor suggests that this is a common pathway for many types of chemically induced and spontaneously occurring tumors. Data were presented which indicate that the ras oncogenes (c-H-ras, c-K-ras, and N-ras) are often associated with human carcinogenesis. Analysis from a variety of human solid tumors indicate that somatic mutational activation of the c-K-ras gene is associated with the development of a major fraction of human pancreatic, colorectal, and lung adenocarcinomas, while activation of the K-ras oncogene is infrequent in other types of carcinomas (breast, kidney, and lung squamous cell carcinomas). Activation of this and other oncogenes may occur either early or late during tumorigenesis where it contributes in a dominant manner to tumor initiation or progression, including the metastatic process. It is striking that the oncogene changes reported in specific types of human tumors in the Japanese population are similar to those reported in the same types of tumors in the American population.

Considerable progress was also made by both Japanese and U.S. researchers in the area of food mutagens. Using *in vitro* mutagenicity testing, mutagens and mutagen precursors have been surveyed in a wide range of foods. A number of mutagen precursors, which are not mutagenic by themselves, but which become mutagenic following treatment with nitrite have been identified. Indole and indole derivatives and several azaimidazoheterocyclic amines became mutagenic in the Salmonella assay without metabolic activation upon nitrite treatment. Several of these mutagens evoked positive responses in an *in vivo* short-term assay for potential stomach carcinogens. U.S. researchers reported that during frying of ground beef, the low molecular weight components responsible for mutagen formation are transported to the surface of the meat where they react at high temperatures to form mutagens. If these water-soluble, low molecular weight components are removed by brief heating in a microwave oven, mutagens are not formed when the beef is subsequently fried. Studies on the relative potency of three of these mutagens (IQ, MeIQ, PhiP) indicated that MeIQ was the most potent mutagen tested in the Salmonella assay and that PhiP was the most abundant mutagen by mass in fried ground beef. Studies at NCI on carcinogenicity testing of IQ in monkeys found a "huge" hepatocellular carcinoma in one high-dose monkey (20mg/kg-5 days/week-25 months). This collaborative area of investigation promises to provide knowledge that may allow for the prevention of cancer and genetic diseases in which diet is a contributing factor.

Understanding the relationship between mutagenicity and carcinogenicity remains a major goal of the Joint Panels. The picture has changed dramatically since the inception of this panel when it was widely believed that "all mutagens are carcinogens." With the accumulation of results from short-term genetic toxicity tests and rodent carcinogenicity tests, it has become clear that many chemicals exhibiting genetic toxicity *in vitro* are not rodent carcinogens and all rodent carcinogens are not *in vitro* and *in vivo* mutagens. During 1987, the U.S. National Toxicology Program (NTP) reported on a study evaluating the genetic toxicity of 73 chemicals for which recent NTP rodent bioassay results were available. Test results from four *in vitro* assay systems (Salmonella, mouse lymphoma, chromosome abberations, and sister chromatid exchanges in Chinese hamster ovary cells) were evaluated for their ability to predict carcinogenicity in the rodent bioassay. The major conclusions drawn from this study are that the results from the four *in vitro* assays did not show significant differences in concordance with the rodent carcinogenicity; i.e., each of the assays has a approximate 60% accuracy. Also, there was no evidence of complementarity among the four assays and no battery of tests constructed from these assays substantially improved on the overall performance of the Salmonella assay. A significant proportion of the carcinogens in this study were not mutagenic.

SIGNIFICANT ACCOMPLISHMENTS

Since the establishment of the Joint Panels in 1972, progress has been made in all areas of the Panel Guidelines. From the very onset, it was clear that one major thrust of this new Panels' activities would be to encourage test method development and validation. Even in 1972, there were numerous methods proposed for use in mass-screening programs that were being designed to test environmental chemicals, but few had been appropriately validated. The Joint Panels played a leading role in developing, assessing, improving, and validating short-term tests to detect mutagenic activity, as well as assays to evaluate the effects of exposure of humans to mutagenic/carcinogenic agents. These tests are now widely used for detecting environmental mutagens and potential carcinogens.

Several panel members also participated in the UNEP/ILO/WHO International Programme on Chemical Safety collaborative study on the evaluation of mutagenicity test systems which were widely used as primary screening methods of potential carcinogenic compounds.

A major development was the establishment of the Environmental Mutagenesis Test Development Program in the National Toxicology Program at NIEHS. This program started in 1979 and was designed for (1) development of an integrated program for routine testing of large numbers of chemicals for mutagenicity which included identification and development of new test systems, increased understanding of the mutagenesis process, test system validation, and development of a strategy for conducting large-scale testing and data evaluation and (2) implementation of a large-scale testing program capable of testing up to 1,000 chemicals per year in microbial mutagenicity assays. One factor that entered into the selection of chemicals for testing was acquisition of genetic toxicology data on chemcials also selected for chronic rodent toxicity studies. An analysis of this data base showed that not all mutagenic chemicals were carcinogenic, and that a significant proportion of the carcinogens were not mutagenic.

In 1972, the same year that this Panel was established, the mutagenicity of AF-2, a major food preservative in Japan (used in such food as soybean curd and fish sausage) was found to be a potent mutagen. Public interest on the mutagenicity of environmental chemicals began to increase. Particularly puzzling at that time was the fact that AF-2 was negative in the Salmonella assay but it was positive in tests with *E. coli* WP 2. Resolution of this important problem, where nearly the entire population of Japan was exposed to this compound, was a focal point of Joint Panel activities during this period. The activity was culminated in a Joint Workshop that was held in Japan in 1974. The meeting led to the development of new mechanisms in Japan for problem definition and resolution in the area of environmental health problems. The discussions that took place at this workshop on the mutagenicity of AF-2 in a wide variety of assay systems as well as its carcinogenicity in new rodent bioassays for cancer conducted in Japan, led to a total ban on the use of AF-2 in 1974.

Another major problem area of interest to the Panel was the discovery in Japan and in the United States of the formation of mutagens in foods processed at high temperatures. The discovery that broiled or pan-fried hamburger contained mutagenic constitutents led to the development of major research programs in both countries to identify and characterize the mutagenic components. Because of the low levels of these mutagens, a major effort was required to obtain them in sufficient quantity for comprehensive mutagenicity and carcinogenicity tests. In the past few years, survey-type experiments using bacterial assays for mutagenicity have revealed the presence of mutagenic chemicals in a wide range of foods and beverages. The potential carcinogenicity of some of these food mutagens has also been shown by bioassays in rodents. In some cases, these mutagens are associated with the way the foods are processed; in other cases, the mutagenicity results from chemicals accumulated by the plants themselves or as the result of fungal contamination and the accumulation of mycotoxins. A number of workshops on this topic have been organized by the panel, and findings have triggered worldwide interest in this research area. Studies on the signficance and risk estimation of mutagens and carcinogens in food are continuing.

To evaluate the consequences of human exposure to environmental mutagens and carcinogens on both somatic cells and germ cells has also been a longstanding problem, and a major focal point of Joint Panel activities. Considerable progress has been in the development of new technologies and methodologies, particularly at the molecular/cellular level, as is indicated in the following examples.

Assays for the analysis of chromosome aberrations in peripheral lymphocytes have been particularly useful and the same cells can be used to measure the frequencies of sister chromatid exchange. Genotoxic exposure can also be identified by measuring DNA (or hemoglobin) adduct levels in peripheral blood samples. Promising new assays to measure the induction of gene mutations in somatic cells continue to be developed, for example, the glycophorin assay on human erythrocytes, which measures the cumulative mutagenic burden of each individual and may be a means to estimate the risk of expressing genetic damage. Chromosome-specific human gene libraries are being produced and distributed to the scientific community for the study and diagnosis of genetic disease.

The findings that oncogenes are activated by carcinogen treatment and that this activation process is causally involved in tumor induction in both humans and rodents have greatly advanced our understanding of the molecular mechanisms of carcinogenesis, and will make the results from rodent carcinogenesis studies more relevant to the assessment of human risk. Similarly, the findings that normal cells contain genes whose products normally suppress growth (tumor-suppressor genes), and that the loss of such genes results in autonomous growth, could have profound implications in terms of pharmacologic approaches to tumor prevention and treatment. The panel will continue to focus on the molecular mechanisms of mutagenesis and carcinogenesis, and expects to continue to address a wide range of interesting and challenging problems in the near future.

PROJECTS SUPPORTED BY THE PROGRAM

Investigator and Institution	*Title*
GRANTS	
Seymour Abrahamson University of Wisconsin	Induced Mutation in Germinal Stem Cells
Bruce Ames University of California	Mutagenicity of Naturally Occurring Chemicals
Stephen E. Bloom Cornell University	Embryos for Detecting Environmental Mutagens
Mary K. Conner University of Pittsburgh	A Model for Persistence of SCE Lesion

Joseph B. Guttenplan New York University	Mechanisms of Carcinogenesis and Mutagenesis
Leonard F. Bjeldanes University of California Berkeley	Mutagens, Carcinogens Produced by *Fusarium Moniliforme*
John H. Burki University of California	Alkylating Carcinogen Mutagenesis in Mammalian Cells
John E. Casida University of California Berkeley	Mutagenic Pesticide Metabolites, Photoproducts, and Impurities
Steven M. D'Ambrosio Ohio State University	DNA Damage Following Exposure to Genotoxins
Steven M. D'Ambrosio Ohio State University	Genotoxicity in Human Fetal Brain, Dermis, and Kidney Cells
Nahum J. Duker Temple University	Molecular Basis for Inhibition of DNA Repair Initiation
Eric Eisenstadt Harvard University	Mutagenic Specificity of Environmental Carcinogens
Eric Eisenstadt Harvard University	Mutagenicity and Metabolism of Cyclopenta (C,D) Pyrene
Michael S. Esposito University of California Berkeley	Genetic Effects of Carcinogens in Mitosis and Meiosis
Darrel P. Falk Syracuse University	Environmental Chemicals and Chromosome Breakage
Mary E. Gaulden University of Texas Dallas	Grasshopper Neuroblast Mutagen Test System
James M. Gentile Hope College	*In Vitro* Activation of Promutagens by Green Plants
Paul R. Gladstone Genetic Systems Corporation	Rapid Methods to Monitor Genetic Damage in Humans
Avram Gold University of North Carolina	Activation of Polycyclic Environmental Mutagens
Arthur Grollman State University of New York	Molecular Toxicology of DNA Adducts
Howard G. Gratzner University of Miami	*In Vitro* and *In Vivo* Mutagenesis Testing by Flow Cytometry
Lawrence Grossman Johns Hopkins University	Human DNA Repair Genes DNA Repair Enzymes in Cells Mutating Antibody Genes
Philip E. Hartman Johns Hopkins University	Detection of Environmental Mutagens and Antimutagens
Ronald G. Harvey University of Chicago	Oligonucleotide Adducts of Carcinogenic Hydrocarbons
Paul Howard Case Western Reserve University	Metabolism and DNA Adducts of Carcinogenic Nitrocarbons

Philip M. Iannaccone Northwestern University	Reproductive Toxicology of Environmental Mutagens
Timothy Jorgensen Johns Hopkins University	Radiation-Induced DNA Damage and Repair at Low Doses
Michael Karin University of Southern California	Interactions of Heavy Metal Ions with the Human Genome
Subodh Kumar SUNY—College at Buffalo	Mutagenicity Studies with Dibenz (A,H) Acridine Derivative
Karl G. Lark University of Utah	Use of Plant Cell Culture to Study Genetic Variation
James M. Lavelle University of Connecticut	Interactions between Metals and Non-Metal Mutagens
Edmond J. LaVoie Naylor Dana Institute	Quinolines: Environmental Mutagens and Carcinogens
William R. Lee Louisiana State University	Genetic Effect of Alkylation in Repair Deficient Oocytes
William R. Lee Louisiana State University	Mechanisms of Mutagenesis for Environmental Mutagens
William R. Lee Louisiana State University	Quantitative Genetic Study of Environmental Mutagens
John H. Peters SRI International	Food-Derived Promutagens/Procarcinogens
Michael J. Plewa University of Illinois	Maize Monitor for Environmental Genotoxins
Louise Prakash University of Rochester	Alkylating Agents: DNA Damage, Repair, and Mutagenesis
Michael S. Risley Fordham University	*In Vitro* Analysis of Genotoxicity on Spermatogenesis
Michael S. Risley Cornell University	*In Vitro* Spermatogenic Cell Genotoxicity Tests
Herbert S. Rosenkranz Case Western Reserve University	Nitroarenes: Mutagenicity and DNA Adducts
Herbert S. Rosenkranz Case Western Reserve University	Studies on a Class of Ubiquitous Environmental Mutagens
Howard G. Shertzer University of Cincinnati	Protection from Environmental Toxins/Mutagens by Indoles
Russell O. Sinnhuber Oregon State University Corvallis	Metabolism and Carcinogenicity of Aflatoxins
Michael J. Smerdon Washington State University	Carcinogen Damage and Repair of DNA in Human Chromatin
Dennis P. Smith Southern Methodist University	Environmental Mutagenesis and DNA Repair

Leland L. Smith University of Texas Medical Branch	Environmentally Induced Mutagenicity
Frederik H. Sobels University of Leiden	Mutation Induction by Genotoxic Agents
Thomas D. Stamato Wistar Institute	Isolating Carcinogen Sensitive Mammalian Cell Mutants
Thomas D. Stamato Wistar Institute	Mutagen Induction of Sectored Colonies in CHO Cells
Reggie H. Stevens University of Iowa	Radiation-Equivalency of Mutagens/Carcinogens
Eric Moon-Shong Tang University of Texas System Cancer	Repair of DNA Damaged by Model Environmental Chemicals Center
Milton Taylor Indiana University	Short-term Mutagenicity Testing with Human and Murine Cells
Robert T. Taylor Lawrence Livermore National Laboratory	Metal Methylation and Mutagenesis
Charles C. Tong American Health Foundation	Comparative Genetic Effects of Environmental Chemicals
David Trkula Baylor College of Medicine	The Role of Herpesvirus Thymidine Kinase in Mutagenesis
Guylyn R. Warren Montana State University	Interaction of Pollutants: Effects on Genetic Toxicity
John D. Wilson Virginia Commonwealth University	Modeling Cellular Response to Genotoxic Agents
Gerald N. Wogan Massachusetts Institute of Technology	Genetic Toxicology Testing
Gerald M. Wogan Massachusetts Institute of Technology	Mutagenic Activity of Nitrosamines
Ronald Yasbin University of Rochester	Excision Repair in *Bacillus Subtilis*

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Japanese Environmental Mutagenesis and Carcinogenesis Panel

BROAD GOALS

Toxic chemicals present in our everyday environment can cause diverse effects on human health. The major health effects of concern to the panel are cancer and heritable genetic diseases. Prevention of the diverse health problems caused by environmental toxic chemicals, environmental mutagens and carcinogens requires their rapid detection and limitation of human exposure. The research activities of the panel have been carried out according to the guidelines that were revised in 1985. These are 1) development, refinement and validation of new methodologies for assessing mutagenic and carcinogenic chemicals, 2) detection of mutagens, carcinogens, tumor-promoters and modifiers of mutagenesis and carcinogenesis in our daily environment using both *in vitro* and *in vivo* assay systems, 3) analysis of biological effects and the mechanisms of environmental mutagens and carcinogens, and 4) development of approaches for human population monitoring.

PROGRESS IN PRIORITY AREAS

A. Development of the short-term assay systems for the environmental mutagens and carcinogens

Several new *in vitro* and *in vivo* short-term assays were developed for detecting environmental mutagens and possible carcinogens. An *in vivo* short-term assay for possible stomach carcinogens was developed by measuring the induction of unscheduled DNA synthesis and DNA strand scission as a marker of tumor-initiating activity and the induction of replicative DNA synthesis (cell proliferation) and ornithine decarboxylase as a marker of tumor-promoting activity on the stomach mucosa, which was exposed *in vivo* with a test compound administered by stomach tube. Using this assay several possible complete stomach carcinogens and potential stomach tumor promoters were identified.

A novel and simple Rec-assay in Drosophila was developed using the DNA-repair deficient male fly which has a double mutation of DNA-repair capacity on X-chromosome and DNA-repair proficient female fly which has an attached X-chromosome having normal DNA repair capacity. All male progenies from this parent have an X-chromosome from the father and are DNA-repair deficient and all female progenies have an attached X-chromosome having normal DNA-repair capacity. Larvae are treated with the test compound and hatched male and female flies are counted. If the survival rate of males was lower than that of females, the test compound is considered a DNA-damaging agent.

An *in vivo* short-term assay for potential liver carcinogens was developed by studying the induction of preneoplastic foci in the liver that were detected by immunochemical staining using antibody against the placenta-type glutathione S-transferase. Male F344 rats were initially given an intraperitoneal injection of diethylnitrosamine followed by partial hepatectomy at the 3rd week. The test compound was administered by diet from the 2nd week to the 8th week. There were good correlations between induction of preneoplastic foci in the liver and liver carcinogens.

New Salmonella test strains having high sensitivity to the nitroarenes and arylamines were developed by the recombination DNA technology. Genes for nitroreductase and acetyltransferase, which are key enzymes for metabolic activation of nitroarenes and arylamines, respectively, were isolated from Salmonella DNA by DNA cloning. These genes were separately incorporated into plasmids and transducted to Salmonella test strains, TA98 and TA100. These new test strains having higher enzymatic activity of nitroreductase or acetyltransferase are useful in monitoring environmental pollution.

B. Detection of mutagens, carcinogens and tumor-promoters in our daily environment

Many different classes of chemical compounds are present in the everyday environment and humans are exposed to these multiple carcinogens. Carcinogenic risk of low-dose exposure to multiple known carcinogens was studied. Forty different carcinogens, whose TD50 values were reported, were given orally into F344 male rats for 102 weeks by mixing them in the diet at the level of 1:50 of each TD50 value. The incidence of liver tumors (hyperplastic nodule and hepatic cell tumor) was higher in the experimental group than that of control group. The incidence of lung tumors and folicular thyroid tumors were also higher than those of control group. Carcinogens having similar target-organ specificity induced tumors additively even at the low-exposure level of small amounts of each carcinogen.

Carcinogenicity of phenolic natural anti-oxidants, caffeic acid, and sesamol, was studied due to the fact that anti-oxidant BHA had induced stomach tumors in rats. These anti-oxidants in powdered diet at a 2% level were given to F344 male rats for 60 weeks. All rats in the experimental groups developed hyperplasia and papilloma in stomach. Continuous feeding of caffeic acid and sesamol induced squamous cell carcinomas in stomach of rats as did BHA. Hyperplasia, which was produced in stomach by feeding of caffeic acid for 24 weeks, disappeared after feeding of a basal diet for 24 more weeks. These preneoplastic changes in the stomach were reversible. Continuous feeding of 0.8% catechol induced adenocarcinomas in the glandular stomach. These anti-oxidants lack mutagenicity and DNA-damaging activity but induce continuous strong cell proliferation which is essential to tumor development by such non-genotoxic carcinogens.

C. Modifiers of mutagenesis and carcinogenesis

An epidemiological survey of human cancer showed the multiple factors in the diet play a role as modifiers of tumor induction. A high-fat diet stimulated, experimentally, the induction of mammary and colo-rectal tumors. 4-Nitroquinoline 1-oxide was given subcutaneously into the back of 6-week old male ICR mice and the mice were given a plain basal diet or a basal diet containing 20% corn oil. Incidence of lung tumors and numbers of lung tumors per mouse in the corn-oil diet group was higher than those of the basal diet control group. A high-fat diet enchanced tumor induction in the lung as well as in the mammary gland, colon/rectum, and pancreas.

Staurosporine is an alkaloid isolated from *Streptomyces sp.* and has a strong inhibitory effect on protein kinase C and also inhibits HL-60 cell adhesion. Staurosporine suppressed tumor promotion by teleocidine in the two-stage skin carcinogenesis initiated by DMBA. This compound was an anti-tumor promoter of the skin carcinogenesis.

(-)-Epigallocatechin gallate was isolated from green tealeaf and showed anti-tumor promoter activity. This compound suppressed tumor incidence in the two-stage skin carcinogenesis of mice initiated by dimethylbenzanthracene and promoted by teleocidin. This compound also inhibited protein kinase C activity and weakly inhibited binding of H-TPA with its receptor.

Sodium chloride is a tumor promoter of stomach carcinogenesis and induces cell proliferation of stomach mucosa, namely, induces replicative DNA synthesis in stomach mucosa.

Co-administration of CaCl at 50-200 mg/kg body weight with NaCl or administration of CaCl 1 hour before NaCl administration suppressed induction of replicative DNA synthesis; namely, cell proliferation induced by NaCl, stomach tumor promoter. Ca ion is the possible anti-tumor promoter of stomach carcinogenesis.

D. Monitoring of humen exposure

Blue-rayon, rayon-bound covalently copper-phthalocyanine, absorbs specifically polycyclic compounds, and the absorbed polycyclic mutagens are eluted with methanol-ammonia and detected by the bacterial mutation test. Blue-rayon was used to monitor mutagens in river water by soaking it in the river stream for more than 24 hours. Blue-rayon is useful for study of mutagens present in drinking water and monitor of mutagens in aquatic environment. Blue-rayon is also useful for isolation of polycyclic mutagens from crude sample or analysis of mutagens and carcinogens in human urine sample.

For monitoring human exposure to environmental mutagens and carcinogens, it is important to detect DNA-adducts formation qualitatively and quantitatively in different organs. The P-Post labeling method was one of suitable assays for this purpose and was applied to detecting DNA-adducts in the liver of rats which were given heterocyclic amines, potent food mutagens and carcinogens. After 24 hours of single administration of 100 mg/kg heterocyclic amines by gavage, liver had 64 adducts per 10^7 nucleotides by Trp-P-1 in maximum and 0.3 adducts by Glu-P-2 in minimum. Imidazoquinoxaline and imidazoquinoxaline-type mutagens produced less DNA-adducts than pyridoindole or dipyridoindole-type mutagens. The P-Post labeling method was also applied to detect DNA-adducts of Mitomycin C in the liver and peripheral leukocytes of a patient who was treated with this drug before an operation.

SIGNIFICANT ACCOMPLISHMENTS

Development of short-term in vivo assay for possible stomach carcinogens

Many *in vitro* short-term assays are developed and widely used for screening environmental mutagens and possible genotoxic carcinogens. However, a good *in vivo* short-term assay is requested to develop and establish for the evaluation *in vivo* biological effects of *in vitro* mutagens and possible genotoxic carcinogens, that are detected by *in vitro* short-term assay. Therefore, an *in vivo* short-term assay was established for detecting possible stomach carcinogens because stomach cancer is a big problem not only in Japan but also in the world.

Test compound is administered into rat by stomach tube and after several incubation periods stomach mucosa is removed and markers of tumor-initiating and -promoting activities are studied on stomach mucosa. For the marker of stomach tumor-initiating activity, inductions of unscheduled DNA synthesis and DNA strand scission are studied *in vitro*. For the marker of stomach tumor-promoting activity, inductions of replicative DNA synthesis (RDS, cell proliferation) and ornithine decarboxylase (ODC) are studied *in vitro*. One test requires 2–3 days and the whole study is completed within 1 month. If test compound shows the induction of both markers of tumor-initiating and -promoting activity, the compound should be possibly genotoxic complete stomach carcinogen, and if test compound induces RDS and ODC, test compound should be possible stomach tumor-promoter. There is a good correlation between the induction of these markers in the stomach mucosa and known stomach carcinogens and stomach tumor-promoters.

By this short-term assay, glyoxal, methyl glyoxal, diacetyl, 1-nitrosoindole-3-acetonitrile, 3-diazo-N-nitrosobamethan, and hickory smoke condensates were predicted as possible complete stomach carcinogens and several sodium and potassium salts, secondary bile acids, formaldehyde, catechol, and 4-methylcatechol are predicted as possible stomach tumor-promoters.

Detecting new tumor promoters in the environment

New TPA-type tumor promoters on mouse skin which are structually different from TPA, 12-O-tetradecanoyl-phorbol-13-acetate, were detected by short-term screening system. This screening system consists of three successive tests, irritation on mouse ear, induction of ornithine decarboxylase (ODC) in mouse skin, and HL-60 cell adhesion. Teleocidins and lyngbyatoxins were isolated from culture medium of fungi and seaweeds, respectively, and have indole structure. Aplysiatoxins were also isolated from seaweeds and have different chemical structure from those of TPA and teleocidins. All these TPA-type tumor promoters have a similar tumor-promoting activity as TPA, and bound to the same receptor as that of TPA.

Palytoxin was non-TPA type of tumor promoter of mouse skin. This is a water soluble toxin isolated from a marine coelenterate. Palytoxin gave strong irritation reaction on mouse ear but did not induce ODC or HL-60 cell adhesion. It showed tumor-promoting activity in a two-stage mouse skin carcinogenesis test. It did not bind to TPA receptor and did not activate protein kinase C.

Another non-TPA type of tumor promoter of mouse skin is Okadaic acid. Okadaic acid was isolated from a black sponge and did not bind with phorbol receptor on cell membrane and did not activate protein kinase C. This compound induced irritation of mouse ear, ODC in mouse skin, and strong tumor-promoting activity in a two-stage mouse skin carcinogenesis test. Okadaic acid bound to serine/threonine protein phosphatase and specifically inhibited phosphatase activity resulting in the apparent increase of phosphoproteins. Therefore, non-TPA type tumor promoters act individually through different mechanisms.

TPA type and non-TPA type tumor promoters act through different mechanisms, but both induce several common biological activities such as irritation on mouse ear and formation of superoxide anion radical. What is a common effect of tumor-promoter and involving tumor-promotion process should be elucidated.

Pyrolysis mutagens and carcinogens in cooked food

Discovery of the formation of mutagens by cooking food presented a unique and novel concept. Smoke condensates from broiled fish and also, charred portions of broiled fish and beef showed mutagenicity in *Salmonella typhimurium* TA98 with metabolic activation. Proteins, starch, DNA, RNA, and fat were pyrolysed and resulting tars were studied for mutagenicity in *S. typhimurium* TA98 and TA100 with and without metabolic activation. Protein pyrolysates showed the highest mutagenicity on both *S. typhimurium* TA98 and TA100 with metabolic activation. Starch pyrolysates showed mutagenicity only on *S. typhimurium* TA100 without metabolic activation. This indicates that at least two different classes of mutagens are produced by heating food constituents; namely, indirect mutagens requiring metabolic activation and direct mutagens not requiring metabolic activation. Therefore, a study of mutagenicity involving pyrolysates of individual amino acids was initiated. Tryptophan pyrolysates produced the highest mutagenicity on *S. typhimurium* TA98 with metabolic activation, followed by serine, glutamic acid, creatinine, creatine, ornithine, and lysine. Mutagens were isolated from the pyrolysis products of 1.0 kg tryptophan at by acid-alkaline partition and several column chromatographies combined with the *Salmonella* mutagenicity assay. Finally, two mutagens, Trp-P-1 (3-amino-1, 4-dimethyl-5H-pyrido[4,3-b]indole) and Trp-2 (3-amino-1-methyl-5H-pyrido(4,3-b]indole) were isolated in crystalline form. Their chemical structures were elucidated by the mass-spectrography, NMR, and X-ray crystallography. Their chemical structures were verified by chemical synthesis and a large-scale chemical synthesis of Trp-P-1 and Trp-P-2 was initiated. Then, carcinogenicities of these two mutagens were determined by animal carcinogenicity tests in mice and rats. Both mutagens produced liver cancer in mice and rats. A series of heterocyclic amine mutagens were isolated quickly from pyrolysates of glutamic acid, lysine, and ornithine and their chemical structures were also elucidated. Glu-P-1 (2-amino-6-methyldipyrido[1.2-alpha,3',2'-d]imidazole) and Glu-P-2 (2 aminodipyrido[1,2-alpha,3'2'-d]imidazole) produced liver and blood vessel tumors in mice and tumors of the liver, intestines, Zymbal gland, and clitoral gland (female) in rats. Two other mutagens were isolated from pyrolysates of soybean globulin.

Another new type of mutagen, amino-imidazoazaarenes, was isolated from charred dried sardines and beef. IQ (2-amino-3-methylimidazo[4,5-f]quinoline and MeIQ (2-amino-3,4-dimethylimidazo[4,5-f] quinoline were isolated from broiled sardine, and IQ and MeIQx (3-amino-3,8-dimethylimidazo[4,5-f]quinozaline) from broiled beef patty. IQ produced tumors in the liver, stomach, and lungs of mice, and tumors in the liver, intestines, Zymbal gland, clitoral gland (female), and skin of rats. MeIQ produced liver and stomach tumors in mice and tumors of the Zymbal gland, oral cavity, colon, skin, and mammary gland (female) in rats. IQ, MeIQ and another two new mutagens 7,8-DiMeIQx and 4,8-DiMeIQx were produced from the mixture of creatinine, amino acids, and saccharides by heating at 135°C in diethyleneglycol-H20. This indicates that creatinine (or creatine), amino acids, and sugars present in fish and meat are precursors of mutagenic amino-imidazoazaarenes.

U.S. scientists started a study of mutagen formation in broiled beef patty. They identified the formation of IQ, MeIQx, and 4,8-DiMeIQx, and isolated new mutagens PhIP, trimethyl-aminoimidazol-pyridine, and two dimethyl-aminoimidazole-pyridines.

Each year at the Joint Panels Conference recent data from this study were exchanged between the two countries and workshops on this topic were held at Livermore, California, on November 6, 1981, and at Honolulu, Hawaii, on December 19, 1984. The exchange of the information stimulated the further progress of this study in both the United States and Japan.

This new field of study was initiated in Japan and developed independently in both countries, but major progress was achieved through cooperative information and resources exchange under the U.S.-Japan Cooperative Medical Science Program.

FUTURE GOALS

For screening genotoxic possible carcinogens, *in vitro* short-term assay is established, but for screening non-genotoxic possible carcinogens *in vitro* short-term assay is limited and need to be developed. *In vivo* short-term assay is required for evaluation of the biological effects of both possible genotoxic and non-genotoxic carcinogens that are detected by *in vitro* short-term assay. *In vivo* short-term assays for genotoxic and non-genotoxic carcinogens of liver and stomach are developed but those assays for other organ-specific carcinogens need to be developed.

Evaluation of relationship between mutagenesis and carcinogenesis and elucidation of the mechanisms of action by environmental mutagens and carcinogens are still important problems, and these projects should be studied further. The studies of oncogene activation and suppressors of oncogenes are progressing. Risk assessments of cancer and inheritable genetic disorder by environmental mutagens and carcinogens are continuous projects to study. Studies on human exposure size to environmental mutagens and carcinogens and development of estimation methodologies on ill-health effects to humans by environmental toxic chemicals are needed for risk assessment.

PROJECTS SUPPORTED BY THE PROGRAM

1. Development and refinement of the bacterial short-term assays, and their application for screening of environmental mutagens.
2. Development and refinement of the *in vitro* short-term assays using mammalian cultured cells, and their application for screening of environmental mutagens.
3. Development and refinement of the *in vivo* short-term assays for screening of environmental mutagens and carcinogens and their application.
4. Development and refinement of the short-term assays for tumor promoters, and their application.
5. Studies on the carcinogenicity of environmental chemicals.
6. Metabolism and detoxication mechanism of environmental mutagens.
7. Development and refinement of monitoring systems of mutagens in our environment and their application.
8. Activation of oncogenes by environmental mutagens and carcinogens.
9. Development and refinement of human monitoring systems on the population exposed to environmental mutagens and carcinogens, and their application.
10. Development and refinement of epidemiological study system on the population exposed to environmental mutagens and carcinogens, and their application.

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Joint Scientific Conferences

ENVIRONMENTAL MUTAGENESIS AND CARCINOGENESIS PANELS

Date: January 8–10, 1985

Place: Shimoda, Japan

Summary: The 12th Joint Conference, dealing with "Mutagenesis and Carcinogenesis by Nitropyrenes and Cancer Chemotherapeutics" was attended by 25 Japanese and 8 U.S. scientists. Sixteen papers were presented in sessions on: 1) mutagenicity of nitropyrenes, 2) carcinogenicity of nitropyrenes, 3) secondary cancer in chemotherapy patients, and 4) genetic toxicology studies in cancer chemotherapy.

Date: October 21–23, 1985

Place: Research Triangle Park, North Carolina

Summary: The 13th Joint Conference on the "Development and Utilization of *in vivo* Short-Term Tests for Mutagenicity and Carcinogenicity" was attended by 5 Japanese and 21 U.S. scientists. Eighteen papers were presented in sessions on: 1) *in vivo* genetic tests, 2) activation of oncogenes, 3) cancer epidemiology, and 4) food mutagen research.

Date: May 27–29, 1986

Place: Nikko, Japan

Summary: The 14th Joint Conference on "Short-Term Test Method Development and Deployment" was attended by 23 Japanese and 8 U.S. scientists. Sixteen papers were presented in sessions on 1) new approaches for utilization of short-term tests, 2) DNA adduct formation, 3) food mutagens, and 4) application of short-term tests to human tissues.

Date: April 14–16, 1987

Place: Livermore, California

Summary: The 15th Joint Conference on "Human Population Monitoring" was attended by 5 Japanese and 17 U.S. scientists. The major emphasis of the Conference was on new methodologies and technologies for detecting genetic effects in humans. Eighteen papers were presented in sessions on: 1) human genome assays, 2) specific locus assays, 3) cytogenetic assays, and 4) correlation of *in vitro* and *in vivo* assays.

Date: July 24–26, 1988

Place: Yokohama, Japan

Summary: The 16th Joint Conference on "Molecular Approaches to Environmental Mutagenesis and Carcinogenesis" was attended by 17 Japanese and 10 U.S. scientists. The Conference was organized to exchange information on several newly emerging areas of research in carcinogenesis and mutagenesis with emphasis on molecular mechanisms. Seventeen papers were presented in sessions on 1) oncogene activation, 2) tumor suppressor genes, 3) tumor promotion, and 4) molecular epidemiology.

Date: February 5–7, 1990

Place: Kailua-Kona, Hawaii

Summary: The 18th Joint Conference on "Non-Genotoxic Carcinogens" was attended by 6 Japanese and 12 U.S. scientists. This Conference focused on non-genotoxic mechanisms involved in the induction of carcinogenesis by various classes of environmental chemicals. Eighteen papers were presented in sessions on: 1) epidemiologic data, 2) model systems: organ sites, and 3) basic mechanisms.

Date: July 16–17, 1990

Place: Tokyo, Japan

Summary: The 19th Joint Conference will be on "Advances in Molecular Mechanisms of Carcinogenesis and Mutagenesis." In honor of the 25th anniversary of the U.S.-Japan Cooperative Medical Sciences Program, past and new Panel members will discuss the influence that concepts and methods in the field of molecular biology are having in the field of environmental mutagenesis and carcinogenesis.

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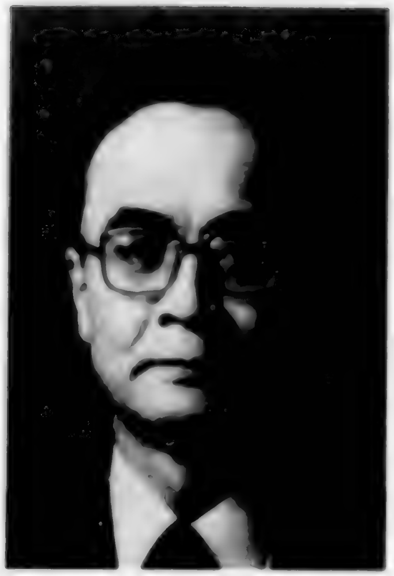

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Profiles

U.S. HEPATITIS PANEL

Dr. Saul Krugman

Chairman (1979–Present)

A graduate of the Medical College of Virginia in 1939, Dr. Krugman trained in pediatrics at the Bellevue Hospital, New York, and for a number of years received research training at that institution under an NIH Fellowship. In 1960, he was appointed to the Chair of Pediatrics at New York University and directed the pediatric service at Bellevue Hospital.

Dr. Krugman is one of the country's distinguished research pediatricians. Without question, he is one of our nation's foremost authorities in the field of hepatitis. His contributions to this important problem are legion. The U.S.-Japan Cooperative Medical Science Program and particularly the Panels on Hepatitis have profited richly from his wise and exemplary advice. He has been one of the most faithful contributing members of the Program and, in spite of his very active and pressing schedule of responsibilities, he has found time to participate and contribute. Always, his comments are direct, concise and much to the point. If the information desired is unavailable, he takes or suggests the important steps needed to answer the question. Scholarly traits and gracious expression are of second nature to him. Like so many others, the U.S.-Japan Program is in his debt.

JAPANESE HEPATITIS PANEL

Dr. Kusuya Nishioka

Chairman (1988–Present)

Following his graduation from the Faculty of Medicine, University of Tokyo in 1947, Dr. Nishioka joined the Institute for Infectious Diseases, University of Tokyo, where he has been engaged in research in the fields of immunology and virology for the past 42 years.

His achievements in the field of immunology have included the discovery of complement components: C3, C5, and C9, and the development of highly sensitive immune adherence hemagglutination tests for detecting antigens and antibodies.

His work, in collaboration with Dr. Okochi and Professor Mayumi, in which a highly sensitive method for detecting hepatitis B surface antigen was set up based on the hemagglutination technique, has resulted in elucidation of the relationship between persistent hepatitis B virus infection and hepatocellular carcinoma.

In 1965, Dr. Nishioka was appointed Chief of the Virology Division of the National Cancer Center. In 1972, he was made Professor of Immunology at the Institute of Medical Science, University of Tokyo. In 1975, he became Vice President of Tokyo Metropolitan Institute of Medical Science and in 1985, Senior Advisor at the Research Center for Liver Diseases, Kitasato Institute. In 1987, he became Vice Director of the Japanese Red Cross Central Blood Center.

During this time, he served as a Member of the WHO Expert Panel in Immunology and Virology and also as Director of the WHO Collaborating Center for Reference and Research on Viral Hepatitis.

Dr. Nishioka has received the title of Honorary Professor of Peking Medical University, PRC, and has greatly promoted international research in the field of viral hepatitis.

He was nominated as Chairman of the Hepatitis Panel for the U.S.-Japan Cooperative Medical Science Program in 1988, succeeding Dr. Oda. For his achievements in complement and viral hepatitis research he was awarded the Japanese Society of Microbiology Award in 1968, the Asahi Prize in 1979, and the Kojima Memorial Award in Infectious Diseases in 1986.

Dr. Toshitsugu Oda

Chairman (1979–1988)

For the past 45 years since he graduated from the Faculty of Medicine, University of Tokyo, Dr. Oda has been engaged in research on liver diseases.

His research in the field of enzymology and virology was initially carried out in the Department of Internal Medicine, University of Tokyo Hospital where he worked until 1982. During his academic life he was nominated as Chairman and Professor of the Department of Internal Medicine (1974) and Dean of Faculty of Medicine (1979). Later he was promoted to Director of the National Medical Center, Japan, and received the title of Professor Emeritus at the University of Tokyo.

At present Dr. Oda is Chairman of the Board for the Japan Society of Hepatology and also Director of the Board for the Japanese Society of Gastroenterology.

When the Hepatitis Panels of the U.S.-Japan Cooperative Medical Science Program were formed, Dr. Oda served as Chairman of the Japanese panel and held this position for 10 years. His input and enthusiasm assured the success of the panel, and he is now dedicated and respected member of the delegation. He established a nationwide cooperative program on the prevention and treatment of viral hepatitis; drawing attention to viral hepatitis B and as a result there has been immense contribution to research in this area.

For his achievements in the field of hepatology research, especially the development of the hepatitis B vaccine, he was awarded the Asahi Prize in 1979 and also was awarded the Purple Ribbon Medal in 1986 by the Japanese Government.

GUIDELINES

HEPATITIS PANELS
United States-Japan Cooperative Medical Science Program

Viral hepatitis is a serious health problem in many areas of the world including Japan, the United States, and Asia. Two known types most commonly recognized are types A and B. Additional types of viral hepatitis have been identified and by exclusion are referred to as non-A non-B hepatitis. Another recently identified agent is the hepatitis delta virus. The long-term objective of the program is to define conditions for the control of viral hepatitis by improved environmental conditions, therapy, or prophylaxis. Research areas of high interest to the program are:

1. Biochemical and biophysical characterization of agents.
2. Seroepidemic studies in Japan, the United States, and Asian countries to identify prevalence and incidence of infections caused by the various hepatitis viruses. Implicit in these objectives is appropriate work on *in vitro* cultivation of hepatitis agents.
3. Development and evaluation of vaccines and antiviral compounds for prevention and treatment of disease with particular emphasis on therapeutic measures for chronic carriers of type B virus.
4. Long-term studies to determine the incidence of cirrhosis and hepatocellular carcinoma as a sequela of chronic viral hepatitis.

U.S. Hepatitis Panel

PROGRESS IN PRIORITY AREAS

The second 5 years continued the rapid progress of the first 5 years in our understanding and control of the hepatitis viruses. A better understanding of the taxonomy of these viruses is emerging and, for purposes of historical perspective and convenience, they have been accorded alphabetical designations. Each virus will be discussed individually.

Hepatitis A Virus (HAV)

During this period, HAV has been molecularly characterized. The genomes of several HAV isolates have been cloned and sequenced. The virus contains a single-stranded, linear, positive sense RNA of approximately 7.5 kb in size. There is surprisingly little genomic heterogeneity among different isolates of HAV. The virus is only distantly related to other picornaviruses and will probably be reclassified from the enterovirus genus to a new genus.

The molecular basis of growth in cell culture, and resultant attenuation, has been examined and epitopes that stimulate neutralizing antibody have been identified and are being characterized. Both inactivated and live, attenuated hepatitis A vaccines have been developed and are currently undergoing clinical testing. The inactivated vaccines, in particular, appear to be practical and should be licensed within the next few years.

Hepatitis B Virus (HBV) and other hepadnaviruses

The genomes of hepadnaviruses are partially double-stranded, circular DNA molecules of only 3.2 kilobase pairs in size, among the smallest of all the animal virus genomes.

Replication of hepatitis B virus has been intensively studied, despite the fact that the virus cannot be isolated and serially propagated in cell culture. Moreover, the related hepatitis viruses of animals, especially the duck hepatitis B virus (DHBV), have been quite useful in studying the replicative strategy of hepadnaviruses in general. The complete replication of DHBV in duck hepatocytes was achieved, providing an important system for determining the replication strategy of the hepadnaviruses. The control of hepadnavirus transcription has been a particular topic of interest. Both *cis*-acting elements (promoters and enhancers) and *trans*-acting elements (products of the X gene and probably parts of the pre-S gene) have proven to be important in regulation of transcription. The discovery of similarities between hepadnaviruses and retroviruses has provided new avenues to the development of antivirals and other therapeutic modalities for hepatitis B.

Plasma-derived hepatitis B vaccines, the success of which was demonstrated during the first 5 years, were replaced with recombinant hepatitis B vaccines derived from yeast and mammalian cells. These vaccines were shown to be at least as immunogenic and protective as plasma-derived vaccines. Despite the wide availability of such vaccines, they appear to have had little impact on the incidence of hepatitis B in the United States, principally because of poor acceptance by the populations at highest risk. Universal vaccination, instead of vaccination of high-risk populations, is being seriously considered in order to improve the impact of these vaccines on public health.

The woodchuck hepatitis virus, the animal hepadnavirus most closely related to HBV, has continued to be a very useful model for studying the natural history of chronic hepatitis. Specifically, the very high incidence of hepatocellular carcinoma among chronically infected woodchucks has made the woodchuck an important model for the study of hepatocellular carcinogenesis. In addition, the woodchuck has proven useful for *in vivo* studies of promising antivirals.

Important new information about the immune response to HBV has been obtained. B cell and T cell epitopes have been identified on nucleocapsid and envelope proteins of HBV by immunization of inbred strains of mice. Strategies are being developed to use this information in the creation of more immunopotent HBV vaccines.

Hepatitis C virus (HCV)

Among the most exciting advances in the study of hepatitis viruses during the past 5 years was the cloning and sequencing of the principal blood-borne non-A non-B hepatitis virus, now designated hepatitis C virus. The virus has been shown to be a small (30-60nm), enveloped virus with a positive sense, single-stranded linear RNA genome of approximately 10,000 kb in size. The virus most closely resembles the flavivirus family of viruses. A serologic test for detecting antibody to the virus (anti-HCV) has been developed and preliminary evaluation has been completed. Based upon this testing, HCV appears to account for 80 to 100% of transfusion-associated non-A non-B hepatitis and at least 60% of community-acquired non-A non-B hepatitis. More importantly the test can detect potentially infectious blood donors in approximately 85% of cases. In contrast, the use of so-called "surrogate tests," such as tests for elevated ALT values or antibody to hepatitis B core antigen, can detect only 30% to 50% of implicated donors. Thus, the test should have a significant impact on the prevention of transfusion-associated non-A non-B hepatitis.

A search is in progress for evidence of additional non-A non-B hepatitis agents. To date, none has been found but the consistent failure to detect anti-HCV in some cases of chronic non-A non-B hepatitis suggests either that additional agents exist or that serologic heterogeneity of HCV strains may be significant. In fact, partial cloning and sequencing of other strains of HCV have revealed a significant genetic heterogeneity among strains, suggesting that screening tests and future vaccines may require broadly reactive antigens.

Hepatitis D virus (HDV)

Hepatitis D virus has proven to be one of the most interesting hepatitis viruses. During this 5-year period, its genome was cloned and sequenced. It is a circular, single-stranded negative sense RNA, only 1.7 kb in size. No mammalian viruses resemble HDV. However, the virus shares similarities and even homology with viroids and virus satellites of plants. All of these agents share similarities with the introns of eukaryotic cells. Thus, they all have significant secondary structure, they share certain core sequences, they all have the ability to autocatalyze cleavage and ligation (''ribozymes''). Clones of HDV have been generated that are infectious by transfection in cell culture systems and in the chimpanzee animal model. Successful transfection in cell culture does not require the helper function of hepadnaviruses that is essential *in vivo* systems.

Hepatitis D virus is an important human pathogen, accounting for up to 50% of the fulminant hepatitis associated with HBV infections and is responsible for rapidly progressive chronic hepatitis in hepatitis B virus carriers who are ''superinfected'' with HDV. Infection with HDV can be prevented by preventing infection with the helper HBV by vaccination, but at present, no means of preventing HDV infection in those already chronically infected with HBV has been found.

Hepatitis E virus (HEV)

The most recently discovered of the human hepatitis viruses is hepatitis E virus. This virus is highly endemic in much of south and central Asia, the Middle East, and north and western Africa and has been responsible for at least three epidemics in Mexico. In regions in which it is endemic, it accounts for about 50% of clinical acute hepatitis.

Hepatitis E virus was successfully transmitted to several primate species. The genome of the virus was cloned and partially sequenced from virus obtained in the bile of an experimentally infected cynomolgus monkey. The genome of the virus appears to be a single-stranded, positive sense linear RNA molecule of approximately 7.6 kb in size. The virus has some of the characteristics of caliciviruses.

Serologic testing for the virus has been hampered by the difficulty of performing immune electronmicroscopy experiments, the only test for antigen and antibody available until recently. However, a new immunofluorescence test was developed that permits more rapid identification of viral antigen in liver and antibody in the serum of humans and experimentally infected primates. Attempts to express viral antigens from recombinant cDNA are in progress.

The epidemiology of HEV is similar to that of HAV. Both are transmitted by fecal-oral means and both have a relatively low mortality (0.1 to 1%) except that HEV produces life-threatening disease in pregnant women. In this group, a mortality of 10% to 20% is common, especially during the third trimester of pregnancy. Preliminary data suggest that normal immune globulin prepared in countries where the virus is endemic may have a protective effect during epidemics. In contrast, normal immune globulin prepared in industrialized countries where the virus is very rare appears to lack protective efficacy. There is a need for active immunity against this important human pathogen.

Therapy for chronic viral hepatitis

Most antivirals have proven to be disappointing in treating viral hepatitis. The most promising has been interferon. Recent studies have demonstrated that recombinant interferons (generally alpha and beta interferons) have provided temporary, and in some cases, permanent improvement in patients with chronic hepatitis caused by each of the three viruses capable of chronic infection HBV, HCV, and HDV. Thus, interferon may to have a place in therapy of such chronic hepatitis.

SIGNIFICANT ACCOMPLISHMENTS

Progress in various priority areas is highlighted in the following summary of achievements during the past 10 years.

Hepatitis A Virus

1. Isolation of the virus in cell culture.
2. Characterization, cloning and sequencing of the genome.
3. Development of candidate vaccines.

Hepatitis B Virus

1. Characterization, cloning, and sequencing of the genome.
2. Development of animal model systems to aid in studies of the pathogenesis and control of HBV.
3. Demonstration of relationships between hepadnaviruses and retroviruses.
4. Development of safe and highly effective plasma-derived and recombinant vaccines.
5. Use of alpha interferon for the treatment of chronic hepatitis B.

Hepatitis C Virus

1. Characterization, cloning, and sequencing of the genome.
2. Beginning characterization of the seroepidemiology of HCV.
3. Demonstration of the association of chronic HCV infection with hepatocellular carcinoma.
4. Development of a screening test for the identification of blood donors who are HCV carriers.

Hepatitis D Virus

1. Discovery and characterization of a fourth human hepatitis agent. The virus is defective and requires coinfection with HBV for its replication.
2. Cloning and sequencing of the viral genome.
3. Demonstration that HDV is similar to viroids and related pathogens of plants.

Hepatitis E Virus

1. Discovery of the fifth human hepatitis virus, associated with water-borne epidemics.
2. Partial characterization, cloning, and sequencing of the genome.

FUTURE GOALS

1. Expanded studies to determine the safety and efficacy of candidate hepatitis A vaccines.
2. Development and implementation of strategies for control of hepatitis B through vaccination.
3. Application of new knowledge of HBV replication for the development of more effective antiviral therapy.
4. Continued monitoring of hepatitis B vaccine efficacy.
5. Continued improvement of tests for serodiagnosis and screening of HCV.
6. Development of vaccines and immunoglobulins for immunoprophylaxis of HCV infection.
7. Development of strategies for the prevention of HDV infection in chronic HBV carriers.
8. Development of a practical serologic test for the identification of HEV infections.
9. Development of vaccines and immunoglobulins for immunoprophylaxis of HEV infection.
10. Search for additional human hepatitis viruses.

Viral hepatitis continues to be a serious health problem in most countries of the world. It is hoped that future advances in hepatitis research will further enhance prospects toward its control.

PROJECTS SUPPORTED BY THE PROGRAM

GRANTS

Investigator and Institution	*Title*
Babbitt, Bruce Liposome Sciences, Inc.	Homogenous assay for hepatitis B virus surface antigen
Bing, David H CBR Laboratories, Inc.	Assays for hepatitis B virus with human leukemic cells
Chisari, Francis V Scripps Clinic and Research Foundation	Pathogenesis of liver disease in hepatitis
Condreay, Lynn D Institute for Cancer Research	Regulation of avian hepadnavirus transcription *in vitro*
Cupps, Thomas R Georgetown University	HBSAG specific immune responses in hepatitis
Dreesman, Gordon R Southwest Foundation for Biomedical Res	Immune response to synthetic HBSAG peptides
Ferrari, Carlo Scripps Clinic and Research Foundation	Immunopathogenesis of type B viral hepatitis
Ganem, Donald E University of California San Francisco	Molecular biology of the hepatitis B-type viruses
Ganem, Donald E University of California San Francisco	Hepatitis B surface antigen secretion and assembly
Garcia, Gabriel Stanford University	Expression vectors of HBSAG in attenuated pathogens
Gerety, Robert Food and Drug Administration	Support for Holloman Air Force Base chimpanzee colony
Halpern, Michael S Wistar Institute of Anatomy and Biology	DHBV infection and associated immunity
Hollinger, F Blaine Baylor College of Medicine	Synthetic hepatitis B vaccine studies

Hollinger, Frederick B Baylor College of Medicine	Delta hepatitis and liver disease in hemophiliacs
Houghton, Michael Chiron Corporation	A diagnostic and vaccine for viral non-A non-B hepatitis
Johnston, Jeffrey M University of Utah	Regulation of adenovirus gene expression in monkey cells
Kennedy, Ronald C Southwest Foundation for Biomedical Res	Idiotypic analysis of the immune response to HBV
Lemon, Stanley M University of North Carolina Chapel Hill	Cloning and sequencing of hepatitis A virus cDNA
Lemon, Stanley M Indiana Foundation	Hepatitis delta infections in hemophiliacs
Lai, Michael M-C University of Southern California	Molecular biology of hepatitis delta virus
Mason, William S Institute for Cancer Research	Hepatitis B-like virus
Milich, David R Scripps Clinic and Research Foundation	MHC-control and cell correlates of HBSAG immune response
Newbold, John E University of North Carolina Chapel Hill	Conventional drugs & antiviral oligonucleotides for HBV
Ou, Jing-Hsiung J University of Southern California	Gene expression and molecular pathogenesis of HBV
McLachlan, Alan Scripps Clinic and Research Foundation	Hepatitis B virus gene expression
Montali, Richard J Smithsonian Institution	Characterization of new primate hepatitis virus
Peeples, Mark E Rush-Presbyterian-St Lukes Medical Ctr	Identification of a cell receptor for hepatitis B virus
Peterson, Darrell L Virginia Commonwealth University	Structure of hepatitis B antigens
Reyes, Gregory R Gene Labs, Inc.	Cloning of non-A non-B hepatitis virus
Robinson, William S Stanford University	The virus of hepatitis B
Robinson, William S Stanford University	A hepatitis virus infected ground squirrel colony
Rosen, Elliot D American Biogenetic Sciences, Inc.	Synthetic vaccines utilizing *Baculovirus polyhedra*
Rutter, William J University of California San Francisco	Molecular analysis of hepatitis B virus
Schloemer, Robert H Indiana Univ-Purdue Univ at Indianapolis	Pathobiology of hepatitis B virus

Seeger, Christopher Cornell University Ithaca	Replication of hepadnaviruses
Sells, Mary A Molecular Diagnostic Systems, Inc.	*In vitro* testing for agents that inhibit HBV replication
Siddiqui, Aleem University of Colorado Hlth Sciences Ctr	Hepatitis B virus DNA as cocarcinogen in kaposi sarcoma
Standring, David N University of California San Francisco	The assembly of HBV particles in xenopus oocytes
Summers, Jesse W Institute for Cancer Research	Replication and pathogenesis of hepatitis B-like viruses
Summers, Donald F University of Utah	Replication and capsid antigens of hepatitis A virus
Taylor, John M Institute for Cancer Research	Structure and replication of hepatitis delta virus
Thanavala, Yasmin Roswell Park Memorial Institute	Clinically relevant models for idiotype HBSAG vaccine
Woolf, Alan Southern Illinois University Carbondale	Factors influencing captive woodchuck breeding success

CONTRACTS

Gerin, John L Georgetown University	Operate molecular virology and immunology laboratory
Phillips, Jere M Meloy Laboratories, Inc.	Facility for nonhuman primates for viral hepatitis
Tennant, Bud C Cornell University Ithaca	Breeding facility for woodchucks (*Marmota mona*)
Vermeulen, Nicholas M J Microprobe Corporation	DNA and RNA extraction from feces for DNA probe analysis
Wright, George E University of Massachusetts Medical School	Antiviral drugs for treatment of human CMV infections

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Japanese Hepatitis Panel

BROAD GOALS

Viral hepatitis is a serious health problem in many areas of the world including Japan, the United States, and Asia. Two known types most commonly recognized are types A and B, which are caused by hepatitis A virus (HAV) and hepatitis B virus (HBV).

Hepatitis type D, which is caused by coinfection or superinfection of HBV and hepatitis D virus (HDV) has been recognized.

Other types of viral hepatitis have been identified and are exclusively referred as non-A non-B hepatitis (NANBH). At least two types have been recognized, one is transmitted by fecal-oral route and its candidate etiological agent is now called hepatitis E virus (HEV). Another one is transmitted via blood and one of the candidate etiological agents is now called hepatitis C virus (HCV).

The long-term objective of the Program is to define conditions for the control of viral hepatitis by improved environmental conditions, therapy, or prophylaxis. During the past decade, preparation of hepatitis B virus vaccine was accomplished. Research areas of high interest to the program are:

1. Biochemical and biophysical characterization of agents including that of NANBH.
2. Seroepidemiologic studies in the United States, Japan, and other Asian countries to identify prevalence and incidence of infections caused by various hepatitis viruses.
3. Development and evaluation of vaccines and antiviral compounds for prevention and treatment of disease with particular emphasis on therapeutic measures for chronic hepatitis of type B virus and non-A non-B. Implicit in these objectives is the appropriate underlying work upon *in vitro* cultivation of hepatitis agents.
4. Long-term studies to determine the incidence of hepatoma as a sequela of chronic viral hepatitis infection.

PROGRESS IN PRIORITY AREAS

1. Hepatitis A Virus Study; Especially the Development of Hepatitis A Vaccine

Hepatitis A vaccines were prepared from HAV strain KRM003 cultured in the African green monkey kidney cell line after purification and inactivation with formalin. This work was done in collaboration with NIH Japan, Chiba Serum Institute, Denka Bio-Research Institute, and the Chemo-Sero-Therapeutic Research Institute.

A Phase I clinical trial was done. Three doses of vaccine ranging from 0.25 mg to 1.0 mg protein antigen were given to 36 volunteers. There was neither untoward side effect nor excretion of HAV from volunteers and a rapid, intense antibody response was observed.

For further development of HAV vaccine, analysis of the antigen-epitope of HAV was conducted to determine the most effective antigen determinants for protection against HAV infection.

2. Hepatitis E Virus as an Etiological Agent of Enterically Transmitted NANBH and its Epidemiology

Shiketa et al., succeeded in experimental infection of cynomologous and rhesus monkeys with a stool extract from Burmese patients and succeeded in the fourth passage in cynomologous monkeys. The causative virus was excreted into the bile and visualized as 27–30 nm particles. This seems to be identical to a putative causative agent of enterically transmitted NANBH as reported by Bradley et al., independently, and designated as hepatitis E virus (HEV).

3. Molecular Biology of HBV

Genomes of HBV were cloned from the plasma of a blood donor who carried subviral particles of three distinct subtypes in the following proportions: adr, 25%; ayr, 63%; and adyr, 12%. HBV DNA clones were classified into two groups based on a difference at only one nucleotide in the S gene. Two clones had A as nucleotide 365 that formed part of the condon for lysine as amino acid residue 122 and produced a surface antigen of subtype adr in transfected NIH 3T3 cells. The remaining four clones had G determining the condon for arginine and produced a surface antigen of subtype ayr in transfected cells. Similarly, HBV genomes were cloned from the plasma of an individual who carried subviral particles of subtypes adr (71%) and adwr (29%). Two clones had T and A as nucleotides 476 and 479, respectively. The other seven clones had C and G as the respective nucleotides. Based on a comparison with previously reported HBV genomes of various subtypes, the mutation of nucleotide 479, forming part of the condon for lysine or arginine as amino acid residue 160, was deduced to determine the w or r subtype, respectively. When NIH 3T3 cells were transfected separately with the genome of subtype adw or adr, derived from plasma containing a surface antigen of subtype adwr, and then cocultured, they produced subviral particles of either subtype adw or adr. When cells were transfected with the genomes of subtypes adw and adr simultaneously, however, subviral particles were produced that possessed w and r determinants on the selfsame particles. These results attributed the d/r and w/r subtypic change to a point mutation in the S gene and favored coinfection of hepatocytes with an HBV genome and its mutant as the mechanism of compound subtypes.

It is generally accepted that HBe antigen positive state in the HBs antigenemia is associated with chronic active hepatitis B and anti-HBe antibody positive state in chronic inactive hepatitis B. On the contrary, chronic active hepatitis B with anti-HBe antibody and HBV DNA in the blood was found in a few cases. By analysis of such cases Akahane et al. found point mutation in the pre-core region at nucleotide 83, tryptophane codon (TGG) to a nonsense codon (TAG) in the genome of HBV from chronic active hepatitis B patients with anti-HBe and high titered HBV DNA. This mutation is responsible for a disturbed secretion of HBeAg into blood and HBe antigen, not being secreted in the blood, accumulated in hepatocytes leading to a poor prognostic course.

Fujisawa et al. succeeded in significant production of L-protein (the product of pre-S1 + pre-S2 + gene) after transferring L-protein gene fused with a chicken lysosomal signal peptide into *Saccharomyces cerevisiae*. The L-protein gene products were found to form typical 23 nm particles containing major spherical and minor tubular particles which produced good antibody response against pre-S1 and pre-S2 and S antigens in rabbit and in BALB/c mice.

4. Development of a New Generation Hepatitis B Vaccine

Based on these molecular biological studies, a new generation HBV vaccine containing the S and pre-S2 domains was developed and the protective efficacy and safety were ascertained in chimpanzees immunized three times with 40–10 mg of vaccines. All chimpanzees gave good antibody response to pre-S2 as well as S antigen and tolerated against challenge with 1,000 infection doses. This candidate vaccine have been tested in human beings as the phase I and phase II studies by Lino et al. Good anti-pre-S2 antibody response ranging to 90% were observed 4 weeks after the second injection with 10 mg im and 20 mg sc administration. The anti-S antibody response was observed 4–8 weeks after the anti-pre-S2 antibody response. The side reactions were mild.

Yoneyama et al., also reported a stable transformed mouse cell line secreting HBs antigen particles containing pre-S2 antigen and the immunological efficacy of antigen particles derived from this cell line was confirmed in H-2 congenic mouse strains.

5. Mother-to-Infant Infection of Hepatitis B Virus and its Prevention

Since the major cause of the development of HBV carrier state in Japan is considered to be a mother-to-infant transmission of the virus, the Ministry of Health and Welfare started a nationwide prevention program in June 1985, and all babies born since January 1986 to HBe-antigen positive carrier mothers have been treated with HBIG and HBV vaccine.

According to this program, the infants born to HBe-antigen positive carrier mothers were injected with 1 ml (200 IU) of HBIG intramuscularly at birth. At the age of 2 months, a second injection of HBIG was given, and at the same time or before 3 months of age, the first dose of HBV vaccine (10 m 1/0.5 ml) was injected subcutaneously. The second and the third doses of HBV vaccine were given at 4 weeks and at 12 weeks after the first injection of HBV vaccine, respectively.

The ratio of the pregnant women who were examined for serum HBsAg was 59% in the first 10 months, and 92% during the next 24 months. The positive rate of serum HBsAG in pregnant women in the latter period was calculated at 1.4%, and that of HBeAg in HBsAg positive women was 24%. The estimated number of HBV carriers developing in babies born in Japan was 4,500/year in 1985, the year before the preventive program began. The number decreased to approximately 800/year in 1987 and 1988.

6. New Developments in the Study of Blood Borne Non-A Non-B Hepatitis Virus (HCV)

Arima reported that 39 plaques specific for hepatitis C infection were cloned. Their size ranged between 63 to 280 bp. Six of them were identical. Those can be divided into three groups according to their antigenic properties tested with the Arima panel reacting with serum obtained from (A) both convalescent and chronic hepatitis C, (B) Convalescent but not with chronic, (C) chronic hepatitis C but not with convalescent.

Seven out of 16 clones showed specific affinities with chronic hepatitis C in the Alter panel: one had 100% affinity and the other two showed 80% affinity with hepatitis C infection. A series of serum obtained serially from two chimpanzees inoculated with the virus showed that the number of positive score to the clones increased by the time passed after the inoculation. RNA reported from six patients with chronic hepatitis C was tested by two clones thus prepared. Positive signals were detected by a preliminary hybridization experiment suggesting a possibility for the genomic diagnosis of hepatitis C infection.

Because a part of cDNA molecularly cloned from plasma of a chimpanzee chronically infected with non-A non-B hepatitis virus was efficiently expressed in yeast, detection of antibodies reacting to the expressed antigen among various hepatitis patients became possible by Kuo et al. in cooperation with Chiron.

The antibody was detected in 15 out of 17 post transfusion non-A non-B hepatitis patients in Japan who later became chronic with multiple alanine aminotransferase (ALT) peaks. Of the 15, 10 patients had received the blood from the donors who were retrospectively shown to be positive for the antibody. In general, the antibody was detected after several peaks of ALT elevations and, once detected, it persisted over years. Among those patients with single ALT peak, however, the antibody was detected in only one patient out of 6.

In acute hepatitis, anti-HCV was positive in 75.6% (31/41) of the post-transfusion and 35.0% (14/40) of sporadic NANBH and only one of 41 type B hepatitis and none of 42 type A hepatitis. The incidence of the progression to chronicity was 80% (36/45) in anti-HCV positive cases showing significant differences at 0.1% level as compared with 25% (9/36) of anti-HCV negative cases.

In chronic liver diseases of NANB type, this antibody was detected in 76.3% (200/262) of chronic hepatitis, 66.7% (106/159) of liver cirrhosis and 76.2% (80/105) of hepatocellular carcinoma. In type B chronic liver diseases it was 5.1% (13/255) in chronic hepatitis, 10.6% (13/123) of liver cirrhosis and 15.8% (12/76) of hepatocellular carcinoma. A history of blood transfusion before development of the diseases was observed in only 3.9% (16/416) of type B chronic liver diseases but was observed in 44.6% (189/424) of non-A non-B type chronic liver diseases who are anti-HCV positive and 33.6% (47/140) of non-A non-B type chronic liver diseases who are anti-HCV negative.

Anti-HCV positivity in blood donors in Tokyo area was 1.2% (34/2,870-. Therefore, screening out of anti-HCV positive blood is now considered to be most potent protection measure not only to post-transfusion NANBGH but also to chronic liver diseases such as chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma of non-A non-B type. Based on these clinical studies, the Japanese Red Cross started screening all blood donors for PT-NANBH using anti-HCV test kit developed by Chiron. This is the world's first nationwide program.

7. Interferon and Chronic Hepatitis Type NANB

To elucidate the dose-dependent effect of interferons on aminotransferases after treatment of chronic hepatitis type NANB, the following studies were carried out by Lino et al.

Study I: Fourteen patients were randomly divided into three groups and IFN-Beta of 1, 3, and 6 mega IU/day were administered to each of 8 groups for 8 weeks. These patients were followed for at least 12 months and were tested for aminotransferases and histological changes of liver tissues.

Study II: Eight patients were randomly allotted to two groups and IFN-alpha (HLBI) in 3 and 6 mega IU/day were intramuscularly injected in each group for 8 weeks. These patients were followed as in Study I.

Study I Results: Serum aminotransferase (ALT and AST) activities decreased rapidly during treatment in all groups and in 4 of 6 patients in 6 mega IU group remained low during the subsequent 12 months of the follow-up period, while reevaluation of ALT was observed in the other two groups. In all three groups, the findings of post-treatment biopsy showed marked improvements in the inflammatory reaction of the lobular and periportal areas and in the 6 mega IU group, the improvement was sustained for over 12 months.

Study II Results: Serum aminotransferase, activities decreased rapidly during treatment in all groups and in two of four patients in 6 mega IU group remained low during the subsequent 6 months of followup, while reevaluation was observed in other groups. In both groups, the findings of post-treatment biopsy showed marked improvements and in the 6 mega IU group the improvement was sustained for over 6 months.

These findings suggest that the higher dose (6 mega IU) administration of interferon for 8 weeks has greater efficacy and these effects may relate to the suppression of replication of non-A non-B hepatitis virus.

SIGNIFICANT ACCOMPLISHMENTS

Immunoprophylaxis of Perinatal Transmission Leading to Persistent Hepatitis B Virus (HBV) Infection

A universal vaccination program, using only HBV vaccine, recommended for all infants by organizations in other countries was judged to be inappropriate for Japan. Instead, an intensive immunoprophylaxis program, in which HBIG and HB vaccinations are given, has been established. This is carried out only on selected high-risk group infants, such as babies born to HBeAg positive mothers, where immunization is effected immediately after birth. This policy is much more effective for the control of HBV persistent infection.

Immunoprophylaxis for HBV infection is quite different from vaccination programs for other infectious diseases, where the spread of infections is universal to babies and cannot be controlled by measures such as improvement of general hygiene. Epidemiological surveys carried out over the past two decades have shown that the greatest source of persistent HBV infection is perinatal infection from HBeAg positive mothers to their neonates. Other mechanisms for transmission of the virus are now greatly reduced. The most effective procedure to prevent perinatal infection of the selected high risk group babies has clearly been adopted. At present, this is combined passive and active immunization of neonates, born to HBeAg positive mothers, at the earliest possible stage, at the latest, 24 hours after birth.

Based on preliminary trials, the Ministry of Health and Welfare in Japan initiated a nationwide prevention program in January 1986. The protocol stipulated that all pregnant women are to be tested for HBsAg and all babies born to HBeAg positive carrier mothers are to be treated with HBIG and HB vaccine.

According to this program, infants born to HBeAg positive mothers are injected with 1 ml (200IU) of HBIG intramuscularly at birth. At the age of 2 months, a second injection of HBIG is given, and at the same time, or at least before 3 months of age, the first dose of HB vaccine (10 ul/0.5 ml) is injected subcutaneously. The second and the third doses of HB vaccine are given at 4 and 12 weeks after the first injection of HB vaccine. The costs for the prevention program are covered by the government.

In 1987, more than 93% of pregnant women in Japan were tested for HBsAg and 23.4% of HBsAg positive mothers were also found to be HBeAg positive. This means that about 5,100 neonates, born to HBeAg positive mothers, received immunoprophylaxis. The protective efficacy is about 95%.

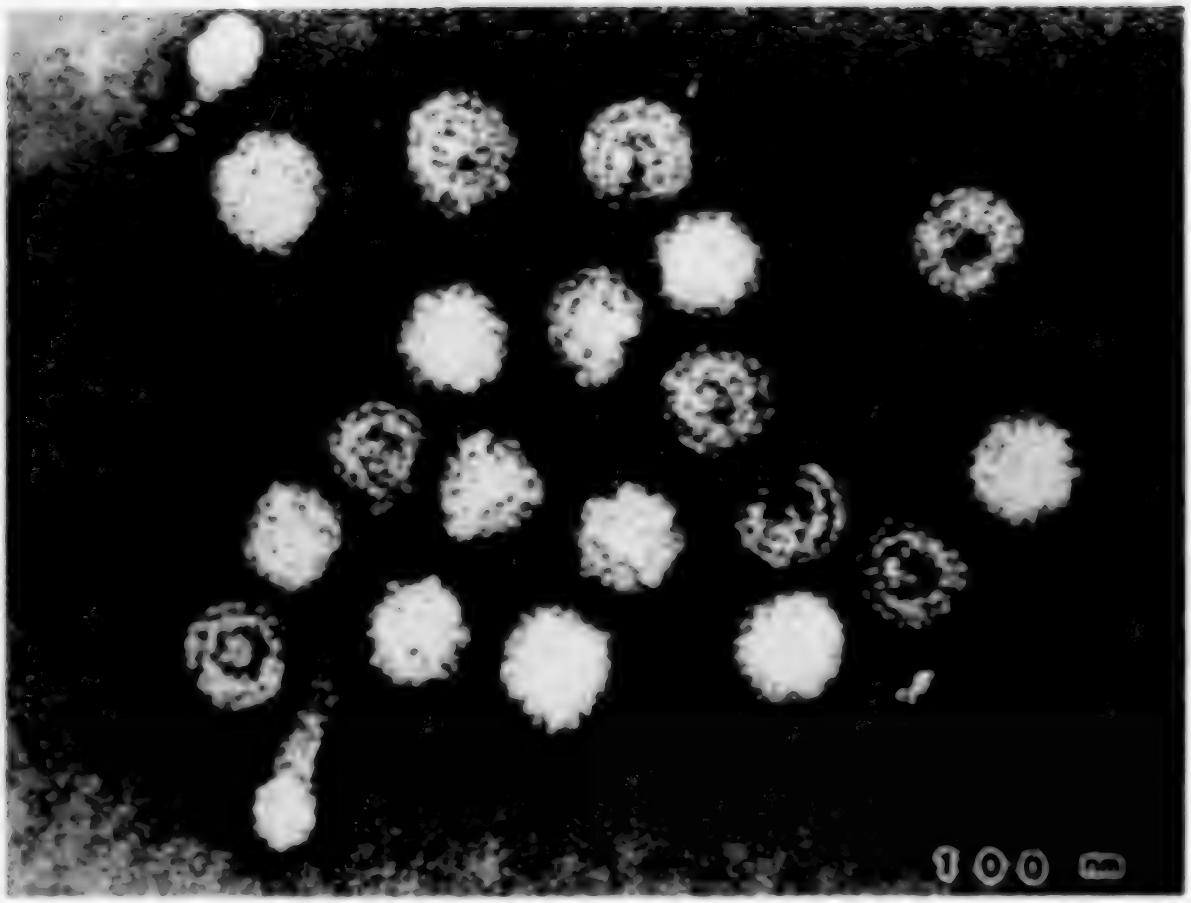
Hepatitis C Virus (HCV) and Hepatocellular Carcinoma

The mortality rate in cases with HBsAg negativity but with liver cancer (HCC), increased significantly from 5.6 in 1968–77, to 12.1 in 1984–85. This is more than double the previous rate, and three-quarters of the total number of HCC cases in recent years. The identification of the causative agent(s), is obviously of prime importance.

Following reports that HCC had developed in individuals with post-transfusion non-A non-B hepatitis (PTNANBH), and that in 40% (43 out of 108) cases with liver cirrhosis and HCC without HBs antigenemia there was a prior history of blood transfusion, attention was drawn to the possibility that a relationship may exist between PTNANBH and liver cancer.

Of the acute hepatitis cases studied; 75% of post-transfusion hepatitis patients were found to be positive for HCVAb and 35% of the sporadic NANB hepatitis patients were positive. Only 1 out of the 41 type B hepatitis patients, and none of the 42 type A hepatitis patients, expressed the HCVAb. The incidence of the progression to chronicity in HCVAb positive cases was 80%, compared with 25% in HCVAb negative cases. This difference is significant at the 0.1% level.

In the cases of chronic liver disease, with NANB type hepatitis, HCVAb was detected in 76% with chronic hepatitis, 66% with liver cirrhosis, and 76% with HCC. However, in the type B chronic liver diseases, the percentages expressing HCVAb were 5% for chronic hepatitis patients, 11% for liver cirrhosis, and 16% for HCC. A history of blood transfusion, prior to developing the disease, was only observed in 5% of HBsAg positive and HCVAb negative liver cancer patients. But, for



Electron micrograph of Hepatitis B virus.

HCVAb positive liver cancer patients 45% were found to have had transfusion at an average of 27 years before developing the disease.

Of blood donors in the Tokyo area, 1.2% (34/2872) were found to be HCVAb positive. Therefore, screening for HCVAb positive blood and subsequent elimination of these units from the pool, which will begin by the end of 1989, should provide effective protection against PTNANBH, and consequently, both liver cirrhosis and HCC, of NANB type, after several decades. These screening tests will be carried out at the Red Cross Blood Centers throughout Japan.

Point Mutation is the S Gene of Hepatitis B Virus

With the advent of recombinant gene technology that disclosed the entire nucleotide sequence of the HBV genome, subtypic variations can now be explained at the amino acid level in the translation product of the S gene and even at the nucleotide level in the sequence of the S gene. It has not yet been possible, however, to pinpoint a single nucleotide substitution in the S gene for the d/y or w/r subtypic change by comparing the sequences reported for HBV genomes of different subtypes.

The four major subtypes have their exceptions. HBsAg of a mixed or compound subtype has been reported that carries both allelic determinants (d and y or w and r), or even all four subtypic determinants, on the same particles. The mechanism whereby such compound particles are created is not yet evident; either genomic recombination of HBV strains of different subtypes or double infection has been proposed to explain them. HBV genomes were propagated from two plasma samples containing HBsAg of subtype adyr or adwr and analyzed their nucleotide sequences. The results indicate a point mutation in the S gene for the d/y or w/r subtypic change and implicate coinfection of hepatocytes with HBV of a certain subtype and its mutant in the production of compound HBsAg particles.

Genomes of HBV were cloned from the plasma of a blood donor who carried subviral particles of three distinct subtypes in the following proportions: adr, 25%; ayr, 63%; and adyr, 12%. HBV DNA clones were classified into two groups based on a difference at only one nucleotide in the S gene. Two clones had A as nucleotide 365 that formed part of the codon for lysine as amino acid residue 122 and produced a surface antigen of subtype adr in transfected NIH 3T3 cells. The remaining four clones had G determining the codon for arginine and produced a surface antigen of subtype ayr in transfected cells. Similarly, HBV genomes were cloned from the plasma of an individual who carried subviral particles of subtypes adr (71%) and adwr (29%). Two clones had T and A as nucleotides 478 and 479, respectively. The other seven clones had C and G as the respective nucleotides. Based on a comparison with previously reported HBV genomes of various subtypes, the mutation of nucleotide 479, forming part of the codon for lysine or arginine as amino acid residue 160, was deduced to determine the w or r subtype, respectively. When NIH 3T3 cells were transfected separately with the genome of subtype adw or adr, derived from plasma containing a surface antigen of subtype adwr, and then co-cultured, they produced subviral particles of either subtype adw or adr. When cells were transfected with the genomes of subtypes adw and adr simultaneously, however, subviral particles were produced that possessed w and r determinants on the selfsame particles. These results attributed the d/y or w/r subtypic change to a point mutation in the S gene and favored co-infection of hepatocytes with an HBV genome and its mutant as the mechanism of compound subtypes.

FUTURE GOALS

It has been recognized that currently among hepatocellular carcinomas (HCC) in Japan, 25% are due to persistent HBV infection and 80% are due to persistent HCV infection. To control these types of infections, (i) elucidation of the mechanism of carcinogenesis of these two different types of viruses, (ii) intervention of progression of the disease process from infection to chronic sequelae, such as liver cirrhosis and hepatocellular carcinoma by antiviral agents, and (iii) development of preventive measures of both types of infections after elucidation of routes of infections are required. Genetical, immunochemical, and molecular biological analysis of HBV and HCV are essentially required. Double infection of two types of the viruses or role of satellite virus such as HDV is also an important problem concerning disease progression. Some 15% of HCC, their etiological factors remain unsolved and further improvement of detection methods of these viruses and participation of other unknown viruses should be pursued.

HBV, HCV, and HBV-associated HDV infections cause acute hepatitis occasionally resulting in fatal infection. Preventive measures by: (i) blocking blood-borne infection, (ii) improvement of vaccination strategies, (iii) elucidation of the mechanism of disease manifestation, and (iv) newer development of treatment are also important projects.

HAV and HEV are now clearly demonstrated as pathogens of enterically transmitted viral hepatitis. Improvement of diagnostic methods and development of vaccines and their evaluation are currently required.

Possibility of the presence of non-A, non-B, non-C, non-D, non-E type of hepatitis viruses of both blood-borne type and enterically transmitted type is an unsolved problem and should be clarified employing newer technologies.

In Asia and Pacific regions, viral hepatitis of these five known types are all present and cause tremendous health problems in these regions. Information exchanges and technology transfer in these regions based on the U.S.-Japan Cooperative Program are expected to contribute to the solution of these problems and should be fortified further.

PROJECTS SUPPORTED BY THE PROGRAM

Research on hepatitis C Virus (HCV).

1. Epidemiological study to identify routes of transmission of HCV.
2. Evaluation of preventive measure for post-transfusion hepatitis C.
3. Analysis of genome and antigen structure of HCV. Comparison of HCV isolates in Japan and U.S.
4. Relation of HCV infection and hepatocarcinogenesis.
5. Determination of chimpanzee infectivity of standard HCV strain for future development of HCV vaccine.
6. Seroepidemiology in Asian countries.

Search for not-identified blood borne hepatitis virus.

1. Experimental infection in chimpanzees.
2. Analysis and followup of post-transfusion hepatitis without HCV markers.

Research on hepatitis B virus (HBV).

1. Molecular pathogenesis of HBV infection.
2. Mutant of HBV and its pathogenesis.
3. Evaluation of preventive measure of perinatal HBV infection.
4. Trial to eradication of post-transfusion hepatitis B, especially fulminant type.
5. Development of new-type HBV vaccine.

Research on hepatitis A virus (HAV).

1. Epidemiological features.
2. Evaluation of HAV vaccine.

Research on hepatitis E virus (HEV).

Epidemiological studies in Asian countries.

Research on hepatitis D virus (HDV).

Epidemiological and clinical survey.

Treatment of hepatitis C and hepatitis B with anti-viral agents.

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Joint Scientific Conferences

HEPATITIS PANELS

Date: March 11–12, 1985

Place: Oiso, Japan

Summary: A total of 24 presentations were made at this meeting—11 and 13, respectively, by U.S. and Japanese participants. There were nine sessions; basic science, pathogenesis, and development and testing of vaccines and antivirals were discussed.

Advances in virology included the cloning and sequencing of hepatitis A virus (HAV). It is classified as an enterovirus.

A recombinant DNA (rDNA) vaccine for HAV is now a possibility. Evaluation of live, attenuated, HAV vaccine candidates shows that finding the appropriate balance between attenuation and significant immunogenicity is difficult. A rDNA hepatitis B virus (HBV) vaccine candidate is safe and immunogenic in humans.

The woodchuck now is a model for acute and chronic HBV and hepatitis delta virus (HDV) infection and disease.

Date: March 7–8, 1986

Place: Williamsburg, Virginia

Summary: A total of 31 presentations were made at this meeting—22 and 9, respectively, by U.S. and Japanese participants. During nine sessions, time was given to basic science, pathogenesis, and development and testing of vaccines, antivirals, and diagnostic reagents.

Characterization of the various hepatitis viruses continues. HAV is not as closely related to the enteroviruses as earlier thought. HAV appears to have a single, immunodominant neutralizing epitope. An improved understanding of replication, assembly and secretion of proteins, immune regulation, and viral integration/hepatocellular carcinoma was evident for hepadnaviruses. Work on HDV indicates an RNA genome, 2 internal proteins and a surface protein borrowed from HBV. Virus-like particles serologically related to enterically transmitted non-A non-B (ETNANB) disease in infected animals and outbreak human cases were reported. The agent appears to be calici-like.

Vaccine development continues for hepatitis A and hepatitis B. A live, attenuated HAV vaccine candidate produced a protective immune response in animal studies. Inactivated vaccine development is at the preclinical stage. Recombinant hepatitis B vaccines are efficacious.

In other areas superinfection of HBV chronic carriers with HDV produces more fulminant disease than does coinfection. Testing of antivirals such as interferon continues. However, little effect is seen when compared to placebo. Evaluation of HBcAg as a surrogate test for post-transfusion non-A non-B (PTNANB) hepatitis showed that screening blood donors in the United States would decrease cases.

Date: March 12–13, 1987

Place: Otsu, Japan

Summary: A total of 33 presentations were made at this meeting—13 and 20, respectively, by U.S. and Japanese participants. There were four sessions and time was given to basic science, epidemiology, pathogenesis, model systems, and development and testing of vaccines and antivirals. Scientific presentations were made on both human immunodeficiency virus and human T cell leukemia virus-1.

Vaccine development continues with a live, attenuated hepatitis A vaccine candidate showing promise. It produced no liver enzyme elevations and most recipients seroconverted. Subunit and synthetic peptide candidates showed very poor immunogenicity. Major questions regarding hepatitis B vaccination are being answered. Boosting of adults appears to be unnecessary since there is a rapid anamnestic response to HBV. Antibody levels and protection are positively correlated to the dose given, making low-dose inoculations inadvisable. *In utero* transmission may be responsible for vaccine failing to prevent perinatal transmission. PreS2 rDNA vaccine candidates for HBV are ready to begin clinical testing.

HBV tissue culture and animal model systems now appear ready for use in antiviral screening.

Basic research advances included: identification of mutations involved in HAV's cell culture adaptation; infection of lymphoid cells prior to entry of woodchuck hepatitis virus into the liver; development of a tissue culture system actively making HBV; production of a monoclonal to HDAg; and identification of HDV a negative-strand virus.

Date: March 16–17, 1988

Place: La Jolla, California

Summary: A total of 34 presentations were made at this meeting—20 and 14, respectively, by U.S. and Japanese participants. There were five sessions and time was given to basic science, seroepidemiology, pathogenesis, development and testing of vaccines, antivirals, and diagnostics.

New approaches to hepatitis B vaccines discussed included synthetic peptides and preS2 subunit approaches. Both elicit protective immune responses in animal models. These were emphasized because of basic research with inbred mice that is unraveling B and T cell responses to various antigens and synthetic peptides. Rapidly growing strains of HAV and more efficient purification procedures are making production for vaccine purposes easier.

Advances were reported on hepadnavirus model systems. The transgenic mouse model permits the study of cause and effect relationships among DNA sequence, gene expression and disease. The woodchuck model has suggested new avenues of research to be explored in humans—the risk of developing HCC after recovery from HBV infection and reactivation of virus from extrahepatic sites after liver transplantation.

Progress in virology includes: construction of infectious nucleic acid from an attenuated strain of HAV; identification of ETNANB disease as associated with one agent; elucidation of enhancer elements and trans-activating factors operating in HBV.

Intravenous drug abuse is causing hepatitis incidence to rise especially in the PTNANB category.

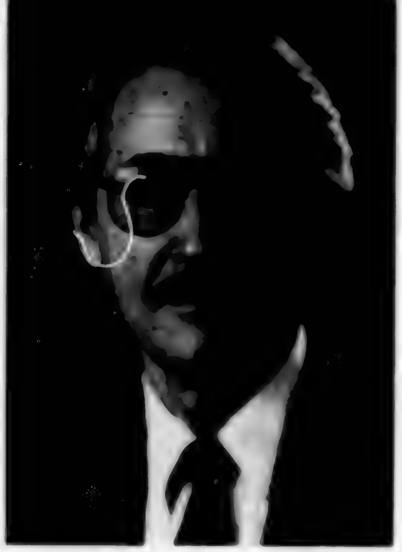
Date: March 16–17, 1989

Place: Gotemba, Japan

Summary: A total of 37 presentations were made at this meeting—17 and 20, respectively, by U.S. and Japanese participants. There were five sessions and time was given to basic science, seroepidemiology, pathogenesis, and development and testing of vaccines, antivirals, and diagnostics.

Progress in basic virology was rapid. Post-transfusion non-A non-B hepatitis virus—hepatitis C virus (HCV) was cloned and sequenced. It is similar to the toga- and flaviviruses. Using a preliminary antibody test there is a focus on epidemiology. Intriguing early data links hepatocellular carcinoma with the presence of antibody making this the second hepatitis virus to be associated with liver cancer. Enterically transmitted NANB (hepatitis E virus, HEV) also has been cloned and partially sequenced.

Vaccine developments still focus on HAV and HBV. Inactivated HAV candidate vaccines are undergoing expanded testing in adults and children. Significant antibody titers are elicited. Pre-S2 rDNA vaccine candidates are safe and tolerable in adults; antibodies to pre-S2 and S were made. Evaluation in non-responder groups is underway.



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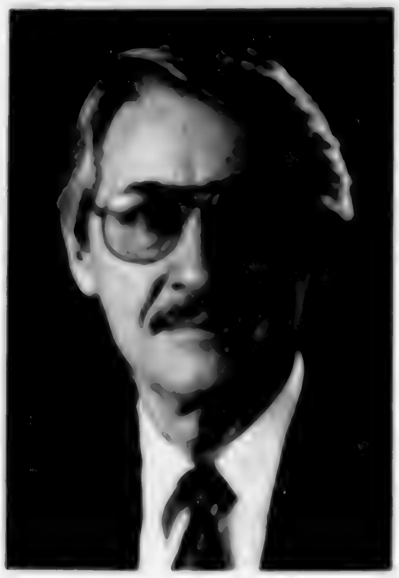
Profiles

U.S. IMMUNOLOGY BOARD



Dr. Max D. Cooper

Chairman (1986–Present)

After graduating from Tulane University Medical School in 1957, Dr. Cooper began his medical career holding appointments in Tulane Medical School, Hospital for Sick Children in London, and the University of California San Francisco Medical Center.

In 1963, he joined the Department of Pediatrics of the University of Minnesota where his research concerned with the dual origins of the immune system in avian species blossomed. He and his associates were the first to establish that the immune system comprised two distinct subsets of lymphocytes, now termed B and T cells, which could be distinguished developmentally and functionally. He has consistently demonstrated his versatility in adapting and applying new information gained in the laboratory to human disorders. His discoveries and insights have provided important advances in understanding and managing immunodeficiency disorders and malignancies such as leukemias.

Currently, Dr. Cooper is Director of the Cellular Immunobiology Unit of the Tumor Institute at the University of Alabama in Birmingham and is also a Howard Hughes Investigator. He is a member of the U.S. National Academy of Sciences. In 1988–89 he was President of the American Association of Immunologists. In 1990, he was the recipient of the 3M Life Sciences Award.

Dr. Cooper is a charter member of the Immunology Board and has served as its Chairman since 1986.

JAPANESE IMMUNOLOGY BOARD

Dr. Tadamitsu Kishimoto
Chairman (1986–Present)

After graduation from the Medical School of Osaka University in 1964, Professor Kishimoto started his research career as an immunologist in the Department of Medicine, Osaka University which was then chaired by Dr. Yamamura. There he demonstrated the pentameric structure of human IgM. In 1970, he became a postdoctoral fellow in the laboratory of Dr. K. Ishizaka at the Johns Hopkins University. His work there showed that the helper function of T cells in the IgM and IgG antibody responses could be mediated by antigen-nonspecific soluble factor(s) released from T cells. This study developed into the discovery of interleukins for B cell regulation, IL-4 and IL-6. After his return to Osaka University, he was promoted to Professor of the Medical School in 1979 and later, Professor of the Institute for Molecular and Cellular Biology. He continued his studies on regulation of B cell responses and discovered IL-6 in 1986. A series of his studies demonstrated that IL-6 plays an important role not only in antibody production but also in hematopoiesis and acute phase reaction. Recently, his group succeeded in the molecular cloning of IL-6 receptor and revealed a unique mechanism of signal transduction through interleukin receptors. His studies have contributed to making "cytokine or interleukin research" one of the most popular fields of immunology.

Dr. Kishimoto has been a member of the Immunology Board since its inception and has served as Chairman since 1986. As a council member of the International Union of Immunological Society (IUIS) as well as the Japanese Immunology Society, he continues to contribute to development of the field of immunology.

Dr. Yuichi Yamamura
Chairman (1981–1985)

After returning from the Navy, Professor Yamamura started his research career in the Department of Organic Chemistry, Faculty of Science, Osaka University, and worked as a medical doctor in the National Sanatorium Toneyama Hospital. At that time, he proved, in rabbits, that experimental tuberculosis cavities were produced by an allergic reaction to the cellular components, especially the protein fraction of the tubercle bacilli, but not the living bacilli. This was not only an important work in tuberculosis but also a pioneer study in the field of cellular immunology. In 1958, Dr. Yamamura was appointed as Professor of Medical Chemistry, Faculty of Medicine, Kyushu University, and then became the Chairman and Professor of Internal Medicine, Osaka University Medical School in 1962. As Professor of Medicine, he expanded his research interests into immunology, hematology, endocrinology, and oncology. He was especially interested in biochemical and immunological studies of tubercle bacilli, and he worked extensively on the application of mycobacterial cell wall skeleton on cancer immunotherapy. He was elected President of Osaka University and held that position from 1980 to 1986.

Dr. Yamamura was a member of the Tuberculosis Panel since it inception and was its chairman from 1978 to 1981. When the Immunology Board was established in 1981, he became its first chairman and served until 1982 when he became a member of the Japanese Delegation. Dr. Yamamura has made a great contribution to the USJCMSP based on his experience in basic and clinical medicine. He is still an active scientist, especially in the field of immunology, and is a leader in the Japanese scientific community.

GUIDELINES

IMMUNOLOGY BOARDS
United States-Japan Cooperative Medical Science Program

Immunology has made rapid advances in the last decade. The recent introduction of the techniques of somatic cell hybridization, molecular genetics, and long-term culture of immunocompetent cells to the study of the immune system has caused an unprecedented increase in our understanding of this field. Such progress in fundamental immunology has the potential to revolutionize the diagnosis, treatment, and prevention of immunologic and infectious disease, including those being studied under the auspices of the U.S.-Japan Cooperative Medical Science Program.

A long-term objective of the Immunology Boards is to study and elucidate the cellular and molecular mechanisms of the immune response and to define effective approaches for the manipulation of the immune system.

In order to achieve these purposes, the Boards will focus their concern on the following research areas:

1. Molecular studies of the genetic organization of the lymphoid system.
2. Cellular and molecular studies of lymphocyte activation, proliferation, and differentiation.
3. Immunogenetic approaches to the study of lymphocyte interactions, with emphasis on the role of histocompatibility gene products in the regulation of the immune response.
4. Cellular and molecular studies of the regulation of the immune response, with emphasis on diagnosis, therapy, and prevention of diseases involving disordered immune function or affected by the immune response.
5. Application of techniques for the development of stable cell lines and of somatic cell hybrids to the study of the regulation of lymphocyte activation and function and to the preparation of monoclonal antibodies, lymphocyte receptors and specific, as well as nonspecific, lymphocyte products.
6. Application of molecular genetics to study functions of the immune system, including genes encoding cell surface antigens, secreted immunoregulatory factors, and important foreign antigens.
7. Mechanisms to support the interaction of immunologists and the exchanges of information and material between the two countries.

7/23/87

U.S. Immunology Board

BROAD GOALS

In 1980, the Joint Committee of the U.S.-Japan Cooperative Medical Science Program sponsored an Immunology Symposium as a first step to adding immunology to the Program. A year later, the Immunology Board was formed. Because this was a discipline, rather than a disease-oriented area, it was named a Board instead of a Panel and funded by the Ministry of Education rather than the Ministry of Health and Welfare.

In reaching the decision to add an Immunology Board, the Joint Committee noted that in the field of immunology rapid advances had been made during the last decade. The recent introduction of techniques of somatic cell hybridization, molecular genetics, and long-term culture of immunocompetent cells applied to studies of the immune system has caused an unprecedented increase in our understanding of this field. Such progress in fundamental immunology has the potential to revolutionize approaches to the diagnosis, treatment, and prevention of immunologic and infectious diseases, including those conditions studied under the auspices of the U.S.-Japan Cooperative Medical Science Program.

Consequently, the Joint Committee agreed that a long-term goal of the Immunology Board should be the study and elucidation of cellular and molecular mechanisms of the immune response and the definition of effective approaches to the manipulation of the immune system.

The goals of the Immunology Boards have been incorporated into the Guidelines presented earlier.

PROGRESS IN PRIORITY AREAS

The U.S.-Japan Immunology Board has been in existence less than 10 years. However, those 10 years have been characterized by the most-rapid rate of advancement ever of new knowledge in the field of immunology. Such astounding progress has been fueled by the development of the techniques and equipment for gene isolation, cloning, sequencing, and transfection, for constructing monoclonal antibodies against virtually any interesting molecule, for enumerating, isolating, and enriching cells present in complex mixtures, and for cultivating and propagating a vast variety of cells *in vitro* under conditions suitable for vigorous analyses. As a consequence, enormous strides have been made in understanding how immune responses are initiated and manifested, and what goes wrong in aberrant or deficient immune responses. New approaches to modifying or selectively diminishing immune responses for prophylactic and therapeutic purposes have been devised.

The past decade might be described as the era of the T cell in immunological research. During that time, the molecular basis of clonal specificity vis-a-vis antigen recognition has been elucidated. The curious histocompatibility restrictions on the interactions of B, T, and antigenpresenting cells have been explained and the molecular details of the specificity restrictions of T-cell receptors clarified. At long last, the remarkable events that occur in the thymus during T-cell differentiation and emigration are yielding to ingenious research: events such as selftolerance, MHC restriction of immunocyte interactions, and the high rate of death of lymphocytes within the thymus.

The past decade might also be described as the era of cytokines. During that time, some 20 distinct molecules that influence the activation, differentiation, and proliferation of leukocytes have been described and characterized and their genes cloned. Many are available in sufficient quantities for therapeutic trials. The specific receptors for most of the cytokines have been characterized and the genes encoding several of them cloned. In general, the cytokines display several biological activities, affecting cells and tissues other than those of the lymphoid system. There remains a great deal of work to be done, including the discovery of new cytokines, but even now it is clear that the usefulness of the cytokines as immunopharmaceutical agents will be enormous.

In the last 5 years, especially, two other areas of immunological research have blossomed (blossomed again would be more accurate); namely, the area of lymphopoietic stem/progenitor cells and the area of antigenic processing and presentation to cognate, responsive lymphocytes. Owing to the fluorescence-activated cell sorting and flow cytometry techniques and the ingenious use of immunodeficient mice (SCID and the triple mutant, bg/nu/xid) the way is now open to definitive qualitative and quantitative studies of both murine and human multipotential and lineage-committed progenitor cells, and their differentiation. With regard to antigen processing and presentation, two remarkable advances stand out: first, the careful tracing of the controlled modifications of protein antigens that result in the preservation of selected peptides which associate with histocompatibility Class II substances to form an immunogenic complex suitable for stimulation of cognate T cells; and, second, the elucidation of the peptide binding site of a Class I histocompatibility substance showing how appropriate antigen fragments may become selectively bound. The implications of these latter two advances remain to be fully appreciated and will be uppermost in the minds of immunologists for the next decade.

Differentiation and Maturation of the Immune System

The generation of the variety of mature blood cells from common precursor cells has long been a favorite system for the analysis of cellular differentiation. The full potential of the system for analysis of lymphocyte differentiation has been realized only recently as the result of several technical advances including: (a) the development of monoclonal antibodies against lineage-associated marker molecules and markers associated with early precursors ("stem cell antigens"); (b) refinements in the apparatus and techniques employed for fluorescenceactivated cell sorting (FACS); (c) improvements in *in vitro* culture procedures that support growth and differentiation of hematolymphoid precursor cells and their progeny; and (d) the development of the mutant, severe-combined-immunodeficient (SCID) mouse and its use in the analysis of development and differentiation of the immune system as well as in diseases of the immune system. All of these techniques and tools have been utilized in the isolation and characterization of the hematolymphoid stem cells of the mouse. The earliest (least mature) of such stem cells occurs with a frequency of about 1 per 2000 cells and by appropriate use of monoclonal antibodies and the FACS apparatus have been separated in virtually pure condition (frequency 1 in 1 to 1 in 2 of separated cell preparation). Thirty of these cells will rescue 50% of lethally irradiated mice from death due to hematopoietic failure and will restore the entire spectrum of blood cells in those mice. In addition to those pluripotential, self-renewable stem cells, other precursor cells have been isolated by use of different combinations of monoclonal antibodies including a pluripotential set lacking self-renewing capacity and several sets of lineage-restricted precursors. Thymic progenitor cells have been isolated directly from the thymus. The least mature of three such types of thymic progenitors has the capacity for extensive self-renewal and can generate all types of thymocytes and T cells.

The immunological repertoire displayed by the T cells in the immunologically mature animal is the product of several closely regulated events. First, the expression and assemblage of the alpha and beta proteins that compose the heterodimeric T cell receptors (TCR). Second, a positive selection process in which developing thymocytes become adapted to recognition of ambient MHC molecules. Third, a negative selection process in which the prospective T cells, capable of reacting so strongly with self-MHC substances as to be potentially dangerous, are eliminated. The specificity repertoire displayed by TCR (each TCR capable of interacting energetically with a limited number of topologically similar processed antigen fragments) is generated by rearrangements of genetic elements to form functional alpha and beta chain genes. For example, a given alpha chain gene may be composed of one of about 15 V gene segments and one of another 50 J segments; additional diversity in the functional gene arises in the joining of V and J segments by variable deletion and/or addition of bases. Construction of a functional beta gene involves rearrangements that result in utilization of one each of about 21 V and 12 J segments. In addition, two D segments are available and a given functional gene may include either, both or neither of them. Again, bases may be added or deleted at the junctions between V and D and D and J segments. In mice, the number of different genes that can be constructed approaches 10^9 or approximately an order of magnitude greater than the number of mature T cells present in the animal.

The positive and negative selection of differentiating thymocytes that occur in the thymus and center around recognition of self-MHC substances are among the most remarkable phenomena in biology. That these processes occur in the thymus is now certain; how they occur from a mechanistic perspective is uncertain. The paradox that thymocytes are both positively and negatively selected for recognition of self-MHC substances particularly needs to be resolved.

Selective amplification of developing thymocytes may depend upon the molecular context in which the TCR interact with ambient MHC molecules. For example, a gene encoding the β chain of a TCR specific for L^d was expressed in a high proportion of $CD8^+$ T cells in $H\text{-}2^b$ transgenic mice; but in $H\text{-}2^k$ mice there were many fewer $CD8^+$ cells expressing the specific TCR. In $H\text{-}2^{b/d}$ mice there were virtually no $CD4^+$ or $CD8^+$ cells that expressed the transgene indicating negative selection against such cells; however, $CD4^-CD8^-$ cells did express the transgene. Thus, the functionally inactive $CD4^-CD8^-$ cells which expressed the transgene were not selected against, whereas potentially autoreactive $CD4^+$ and $CD8^+$ cells were. Such findings indicate that the MHC environment in which thymocytes differentiate distinctly influences the T cell repertoire through both positive and negative selection processes.

The CD8 and CD4 molecules present on T cells interact with nonpolymorphic regions of class I and class II MHC molecules, respectively, and the interactions strengthen the binding of the TCR to their cognate ligands. It has been proposed, therefore, that the interaction of TCR with class I or class II thymus MHC antigens directs the differentiation of immature $CD4^+CD8^+$ into mature $CD4^-8^+$ or $CD4^+8^-$ T cells, respectively.

The molecular structure with which the TCR interacts is created by the binding of antigenic peptides to MHC molecules. The topology of the native MHC molecule is such that the peptide-binding site involves amino acid residues in each of the variable segments of the αβ TCR. Thus, in the limiting situation where the spectrum of TCR is presented with a specific peptide-MHC combination, only a few clones of T cells are prepared to recognize that combination. In the more general case, where the immune system is presented with a complex antigen from which a number of peptides can be generated capable of interacting with a wide variety of products of MHC alleles, many T cells displaying a variety of specific TCR will be engaged in the immunological response. Allogeneic MHC probably confront the immune system with a variety of distinguishable ligands because any given allogeneic MHC product is apt to interact with a spectrum of host self-peptides, thus, stimulating a large number of T cells. As many as 1% of the T cells in a given individual can recognize an allogeneic MHC protein.

There are several substances, termed "self-antigens," which in combination with the products of particular H-2 alleles of the mouse, form ligands that are recognized by nearly all T cells bearing αβ TCR in which the β protein includes Vβ segments of a given family. These self-antigens are referred to as "superantigens." For example, T cells that display Vβ 17a specific for an, as yet unidentified, B cell substance in combination with the H-2 class II molecule, IE. Therefore, T cells expressing the Vβ 17a are completely absent in mice lacking IE that have been infused with bone marrow cells from IE donors. Similarly, T cells displaying Vβ 8.1 or Vβ 6 react to products of the $Mls\text{-}1^a$ locus in combination with any of several class II substances. These T cells are eliminated during the development of self-tolerance in mice possessing the appropriate combination of H-2 haplotype and self-antigen.

A fascinating pair of foreign superantigens are Staphylococcal enterotoxins A and B (SEA, SEB). SEA in combination with MHC substances of most IE and IA haplotypes, interacts with T cells displaying Vβ 1, Vβ 3, and Vβ 11. SEB in combination with products of most IA and IE haplotypes is recognized by TCR containing Vβ 3, Vβ 8.1, Vβ 8.2, and Vβ 8.3. Injecting SEB into neonatal mice results in tolerance that eliminates T cells expressing Vβ 3 and Vβ 8 elements accompanied by a slight compensatory rise in T cells displaying Vβ 6.

Superantigens interact with a significant portion of the T cell repertoire as a result of being recognized in association with MHC products by, usually, a variable element in the αβ TCR, generally Vβ. Because different mouse strains carry between 11 and 21 different Vβ elements, superantigen-induced tolerance could dramatically alter the repertoire of mature T cells in mice.

Studies of human thymocyte differentiation have been carried out by implanting human tissues into SCID mice. Small pieces of human fetal thymus inserted under the kidney capsule of SCID mice generate sizable pieces of thymic tissue. In SCID mice that received a graft of human fetal thymus tissue under the capsule of one kidney and a graft of human fetal liver to the other kidney, human T cells were generated for many weeks. These mice suffered no graft-vs-host disease and were protected against *Pneumocystis carinii* infection. Presumably the immune response against infection was all-human involving antigenpresenting cells derived from the liver graft. These SCID/human (SCID-hu) mice were susceptible to infection with human immunodeficiency virus (HIV). When HIV was inoculated into the thymus graft it could be shown that the virus was present in CD^{4+} cells and the formation of typical syncytia was observed.

The maturation of B cells from their progenitors involves rearrangements of the germline genes preparatory to the biosynthesis of H and L Ig chains. Evidence has supported the view that this is an orderly process in which the expression of mu H chains precedes the expression of L chains during the conversion of preB cells to B cells. A model of the process envisions a dual role for the mu chain that is produced following V-D-J gene rearrangement: first, in terminating further H chain gene rearrangement, thus, insuring allelic exclusion and, second, in inducing L chain gene assembly. In order to test this model, an analysis of stages in the maturation of human B cells from their precursors was carried out. Transformation of human B cell precursors present in fetal bone marrow (depleted of B cells) was accomplished by treatment with Epstein-Barr virus (EBV). The Ig genes in EBV transformants are stable and do not undergo further rearrangements during *in vitro* proliferation. Thus, the condition of the Ig genes at the time of transformation becomes fixed. Among a large number of transformed B cell precursors, novel types of cells were found frequently in which χ or light chains were expressed in the absence of H chain expression. This result was verified both by immunofluorescence analysis of cells using specific monoclonal antibodies and by analysis of Ig gene rearrangement using selective gene segment probes. A number of possible explanations for the existence of the pre-B cells that express only light chain genes has been considered. The most reasonable conclusion appears to be that these cells represent a normal phase in the pathway of B cell development.

The molecular events underlying the recombination of variable genetic elements to form immunoglobulin (Ig) and TCR genes, together with the molecular controls of initiation and rates of transcription of those genes, represent some of the most challenging problems in biology. Research activity in this area is intense and a great deal of information is being generated.

In both T and B cells the recombining of the variable genetic elements involves a system of enzymes termed "recombinases." One mechanism that may moderate Ig and TCR variable gene rearrangements in different tissues or stages of cell differentiation is the accessibility of V, D, and J gene segments to the recombinases. Since the initiation of transcription depends on enhancer elements, the accessibility of the latter to recombinases might constitute a major control mechanism. To test the idea, a gene construct called a "recombination substrate" composed of a Cu gene, with or without the Ig H-chain enhancer, separated from TCR Jβ, Dβ, and Vβ segments situated upstream, was introduced as a transgene into mice. Lacking the enhancer, no evidence of rearrangement in the gene construct was found in any tissue. With the enhancer present, Dβ to Jβ rearrangements occurred in B cells while Dβ to Jβ and Vβ to Dβ Jβ rearrangements occurred only in T cells. It appeared that the presence of the enhancer was necessary for access of the recombinases to the locus while some other element associated with the Vβ segment, possibly the promoter, controlled the T or B cell pattern of Vβ to DJβ rearrangement.

Accessibility of nucleotide sequences located in critical switch (S) regions to recombinases may be a major factor in regulating Ig class (isotype) switching. In class switching, a deletion type of mechanism, which is distinct from the V region assembly process, appears to involve recognition of a set of repeated sequences (S regions) situated 5′ with respect to each set of C region axons. Abelson leukemia virus transformed B cells are frequently used to study the process. Transformants that undergo frequent μ-to- 2b switching when treated with mitogen express switch region recombinase activity. Introduction of a recombination substrate into such cells can be used to demonstrate joining of μ and 2b S regions. Studies such as this have led to the conclusion that heavy chain class switching is achieved by accessibility of C region loci to a common S region recombinase. Transcription of these regions is associated with the switching process. Class switching can be induced in B lymphocytes by mitogens and certain antigens. For example, stimulation with LPS generally results in progeny producing IgM, IgG3, and IgG2b. But when IL-4 is combined with LPS a high frequency of progeny express IgG1. The addition of IL-4 abolishes the production of germline 2b transcripts suggesting that it may alter accessibility of recombinases to the S region of 2b.

Initiation and Generation of Immune Responses

The details of antigen processing and interaction with Class II (Ia) molecules within antigenprocessing cells (APC) are, at last, yielding to analysis. Studies have been performed with well-defined protein antigens and with natural immunogens (monocytogenes). In order to initiate immune responses, both have to be internalized by APC and subjected to biochemical processing. The latter occurs in acidic intracellular vesicles and involves both unfolding and fragmentation of proteins. Appropriate fragments quickly become associated with local Ia molecules and subsequently displayed at the APC surface. The binding of known immunogenic fragments to Ia molecules appears typical of ligand-receptor interactions; e.g., binding to Ia molecules is saturable and is characterized by dissociation constants (Kd) in the micromolar range. It appears that binding of a peptide within the site ("trough") on Ia molecules results in a change in the conformation of the peptide thus creating a new and stable "conformer." The efficiency with which APC present these immunogenic conformers to cognate T cells is controlled in part by the availability of Ia molecules within

APC. In the case of macrophages (MP) as APC, the density of Ia is, in part, regulated by an Iainducing lymphokine secreted by T cells. Conversely, MP elaborate the cytokine IL-1 which indirectly regulates the proliferation of many T cells. Thus, there is a subtle interplay between MP and T cells that is critical in the initiation, and in determining the magnitude, of T cell responses. Recently, it has become clear that there are two subsets of T helper cells that can be readily distinguished (in the mouse, at least) by the spectrum of lymphokines that they synthesize. To illustrate: Th1 cells produce IL-2 and IFN- while Th2 cells generate IL-4 and IL-5. Some lymphokines, such as IL-3 and GMCSF, are formed by both subsets of Th cells.

There is convincing evidence that direct cell-to-cell contact between T lymphocytes and APC is required in the early initiation of immunological responses. These interaction, like a variety of other cell-cell interactions, appear to be promoted by a family of related glycoproteins termed "lymphocyte function-associated" (LFA) molecules. Thus, molecules of LFA-1 are associated with Th cells and the corresponding ligands, ICAM-1 (for "intercellular adhesion molecule"), are found on APC. Similarly, LFA-3 is associated with APC (dendritic cells, for example) and the ligand, CD2, is a well-known molecule located on T cells. Associated interactions between these pairs of complementary molecules greatly enhance the formation of stable conjugates of T cells and APC by significantly reducing the free energy beyond that attributable to TcR-Ia-antigen interaction. The available evidence strongly suggests, but does not yet prove, a role for LFA molecules in T cell-APC and T cell-B cell conjugate formation.

One of the most fascinating and potentially important lines of investigation in recent years has been dedicated to cytokines. These remarkable molecules and their cognate receptors are intimately involved in the regulation of immune responses. The various interleukins, for example, are in part, responsible for up-regulating various receptors, activating B and T cells and macrophages, stimulating growth and/or differentiation of B and T cells, selectively activating genes, stimulating the formation of reactive oxygen free-radicals, triggering immunoglobulin isotype switching and many other effects. A variety of cytokines and their receptors have been characterized, including at least nine interleukins, and in most cases their genes cloned. In addition to their immunoregulatory properties, the cytokines display a variety of other biological activities. Indeed, these other activities ("side-effects") may limit the therapeutic utilization of cytokines in the treatment of immunological diseases.

Molecular Immunology and Disease

The CD4 (L3T4) and CD8 (Lyt2) surface molecules are present on two different subsets of mature T cells but are co-expressed on less mature thymocytes. It appears that these molecules are instrumental in the selective interactions of the two types of T cells with the appropriate MHC molecules. In the case of CD4$^+$ cells, the TCR characteristically interact with Ag fragments associated with Class II molecules while the TCR of CD8$^+$ cells interact with Ag associated with Class I molecules. This selective adhesion of cells is promoted by the affinity between CD4 and Class II molecules and between CD8 and Class I molecules. A cell-cell binding assay was developed to study the interaction between CD8 and Class I molecules in the absence of TCR. The CD8 gene was transfected into CHO cells leading to the expression of CD8 molecules at the cell surface. The binding to monolayers of CD8-expressing CHO cells of other, B lymphoblastoid cells (known to express significant amounts of surface, Class I molecules) was assessed. In this way, it was shown that: (a) the extent of interaction is proportional to the surface density of Class I molecules, (b) the extent of interaction is influenced by the HLA Class I allele involved, and (c) interaction with CD8 molecules appears to be through the 3 domain of the Class I molecule although the 2 chain may also be involved.

It has been assumed that the CD4 and CD8 molecules perform roughly the same function with respect to activation of the T cells that bear them. A test of this assumption was conducted by studying the effects on surface density and distribution of cells activated with the phorbol ester, PMA. The latter exerts its effect by activating protein kinase C (PKC). PMA treatment caused a rapid down-regulation of surface CD4 and TCR molecules both in cells expressing CD4 only and in CD4$^+$ CD8$^+$ cells. In contrast, there was no change in the expression of CD8 molecules. These results suggest that the surface display of CD4 and CD8 molecules is controlled by different intracellular mechanisms.

Currently, there is a great interest in the CD4 molecule owing to its high affinity of interaction with the gp120 envelope glycoprotein of the human immunodeficiency virus (HIV). Attempts to block this interaction with antibodies prepared against gp120 have not been very successful in part because they are weakly neutralizing and in part due to the extensive polymorphism of gp120. On the other hand, monoclonal antibodies against human CD4 do block infection. HIV gp120 interacts weakly, if at all, with murine CD4 in contrast to human CD4. This difference has been exploited in order to ascertain which domain(s) of human CD4 are involved in the binding to gp120. The CD4 molecule is composed of 6 domains: 4 external, 1 transmembrane, and 1 cytoplasmic tail. Chimaeric gene constructs in which the different domains of the human and mouse CD4 genes were linked together in different combinations, were prepared and transfected into CHO cells. The cells were analyzed for surface expression of the domains encoded by the chimaeric genes and for binding of gp120. These experiments demonstrated that amino acid residues within the amino terminal, immunoglobulin-like domain of human CD4 are involved in binding gp120 as well as many of the anti-CD4 monoclonal antibodies that have been prepared.

It is probable that the rules governing epitome recognition by antibody-combining sites are quite different from those controlling recognition by TCR. Clearly, the latter is a much more complicated process requiring, among other things, molecular MHC compatibility. Molecular variations in immunological recognition ultimately must be weighed heavily in regard to autoimmune phenomena. A particularly good illustration of this comes from recent work on T cell induction of experimental autoimmune encephalomyelitis (EAE) in mice. Myelin basic protein (MBP) induces EAE when inoculated into mice with an appropriate adjuvant. It is particularly interesting that different segments of the molecule induce EAE in different strains of mice. For example, a sequence of 37 amino acids at the amino terminus of the molecule is quite effective in inducing the disease in mice of strains PL/J, A/J and BIO.BL. By comparison, the sequence from positions 89–109 (approximately) is most effective in SJL and other strains. Different strains of mice were immunized with MBP and their T cells recovered when EAE appeared. The spectra of specificities of the T cells of several strains toward a panel of 16 different peptides prepared from MBP were determined. The results from two strains are illustrative of the findings: recognition of a nonapeptide at the amino terminus clearly dominated among T cells from B1O.BL mice while T cells from SJL mice were disposed to recognize a peptide including positions 81-114. Presumably, dominance of a peptide with respect to T cell recognition is a reflection of both antigen processing and affinity for certain Class II molecules. From the population of $CD4^+$ T cells that recognized peptide 1-9, 33 clones were established and analyzed for $V\alpha$ and $V\beta$ gene usage. The results revealed that only four of the available pool of $V\alpha$ and $V\beta$ genes were used. This represents incredible selection. Results such as these have cleared the way to preparing monoclonal antibodies and even cloned T cells specific for epitopes associated with the $V\beta$ elements represented on autoaggressive T cells reactive with MBP. Such antibodies and T cell clones have proven to be effective in treating EAE in animals. A similar approach to treating multiple sclerosis in humans looks quite promising.

Evidence that LFA substances are involved in the effector phases of immune phenomena is unequivocal. For example, target cell killing by cytotoxic T lymphocytes, by large granular lymphocytes functioning either in the NK or antibody-facilitated (ADCC) mode involves LFA-1-promoted adhesion. This can be shown clearly by the fact that MAb against LFA-1 selectively block target cell killing. The LFA molecules and their ligands are widely distributed on different cells. Accordingly, they are intimately involved in inflammatory reactions. Three members of the LFA family, LFA-1, Mac-1, and p150,95 are readily located at sites of inflammation. These three molecules are heterodimers that possess identical chains but different (although related) chains. Mac-1, and p150,95 promote adhesion of granulocytes and monocytes to endothelial cells and other tissue substrates. Mac-1 functions as complement receptor type 3 (CR3) and binds iC3b. Both Mac-1 and p150,95 molecules are present in cytoplasmic pools in monocytes and granulocytes. Chemoattractant substances promote the mobilization of these preformed molecules to the surface of the cells with the result that the cells adhere to other cells and substrates at sites of inflammation.

A genetic disease called leukocyte adhesion deficiency (LAD), transmitted as an autosomal recessive condition, results from a deficiency of the β chain of LAF-1, Mac-1, and p150,95. Patients who suffer from LAD experience recurrent, life-threatening bacterial and fungal infections along with other problems. Leukocytes from these patients fail to perform adherence-dependent immune functions.

Recently, the molecule ICAM-1 has been demonstrated to function as a receptor for the influenza virus. Monoclonal antibody against ICAM-1 blocks the entrance of the virus into host cells. This finding may pave the way to effective prophylaxis for influenza.

SIGNIFICANT ACCOMPLISHMENTS

As described in the section on Progress in Priority Areas, it is evident that rapid advances in recombinant technology and genetic engineering have accelerated immunological research by the board.

The success in molecular cloning of both IL-6 and IL-6 receptor prompted search for its possible association with several diseases of unknown etiology and resulted in discovery of its involvements in rheumatoid arthritis and oncogenesis of B lineage cells. Human IL-2 and IL-2R transgenic mice provided an ideal model to delineate their *in vivo* functions for future exploration.

Comparative studies on closely related HLA class I genes and molecules gave an insight into the evolutional pathway and genetic mechanisms by which the current diversity of their specificities were evolved, notably recombinational events in and around the exon 2 of the HLA class I genes. Identification of human minor histocompatibility antigens and their restriction molecules for T cell recognition would warrant further exploration of the mechanisms involved in T cell recognition of allopeptide.

Studies on immune regulation in humans have resulted in discovery of HLA-linked immune suppression gene(s); while studies of I-J peptide and its role in immune regulation have been progressing. Information gained in these fields will undoubtedly contribute to a better understanding of regulatory mechanisms of the immune system and consequently to prevention and control of immunological disorders.

Last but not least, discussions between Japanese and U.S. Board members have been extensive at each of four annual Board-sponsored meetings on either side of the Pacific. All of these meetings have been a very effective means for exchange of information and simultaneously for stimulation of the young immunologists of both countries who joined the meetings.

MAJOR ACHIEVEMENTS

Since its inception in 1981, the U.S. Immunology Board has been composed of leaders in advancing the field of immunology through their research. Some, but by no means all, of the advances that has emanated from their laboratories include the following:

a. The discovery and characterization of new cytokines that affect B lymphocyte differentiation, selective gene activation and immunoglobulin isotype expression.

b. Analysis of factors that influence the synthesis, secretion, and localization of immunoglobulin E antibodies involved in allergic reactions.

c. Preparation of novel immunotoxins and immune response modulating factors and analysis of their effects in depressing or stimulating immune responses in both animal models and clinical trials.

d. Systematic investigations of development and differentiation of the immune system, especially lymphocytes of the B and T cell lineage in both animals models and humans.

e. Studies of the frequencies, phenotypes, and differentiative potentialities of hematopoietic progenitor cells together with the development of methods for isolating progenitor cells and studying their potentialities in immuno-destitute, murine model systems.

f. Investigations of the molecular-genetic basis of antibody and T cell specificities and the genetic organization and evolution of the immunoglobulin superfamily of genes.

g. Identification and characterization of T cell receptors, pioneering investigations of the genetic control of T cell receptor specificity, and seminal analyses of the molecular bases of T cell selection in the thymus.

h. Development and utilization of novel procedures for analyzing the processing, i.e., the controlled fragmentation of protein antigens within the cytosol of antigen processing/presenting cells along with pioneering studies of the formation of immunogenic peptide-Class II molecular complexes within those cells.

It is apparent that the research interests and efforts of the U.S. Board members represents a cross-section of the conceptual and technical fabric of modern immunology. It is equally true that the members of the Board are world leaders in immunological research.

One of the major accomplishments of the Immunology Board—one of long-range significance—has been the creation of cooperative research programs involving members of both the U.S. and Japanese Boards. These collaborative programs are important for two reasons: first, because they result in research of the highest quality, and, second, because they provide the opportunity for postdoctoral fellows to obtain outstanding training and experience.

The greatest accomplishment of the U.S. Immunology Board has been the participation of its members in the joint scientific meetings with the Japanese Immunology Board. Visitors who have attended one or more of these meetings without exception have remarked that they are among the very best meetings in the field of immunology held anywhere. The Immunology Board is attempting to meet periodically with an appropriate Panel in order to exchange information and ideas, thus benefitting both groups of scientists. In December 1987, the Immunology Board and the U.S.-Japan Virology Panel met jointly in Monterey, California. The next meeting will be held jointly with the new AIDS Panel in New Orleans in January 1990.

PROJECTS SUPPORTED BY THE PROGRAM

1. Organization, rearrangement, expression, and evolution of genes encoding molecules of immunological importance (e.g., immunoglobulins, T cell receptors, cytokines receptors).
2. Characterization of cytokines, cytokine receptors, and studies on mechanisms of activity.
3. Development and differentiation of lymphoid cells, control of immunoglobulin synthesis, mechanisms of immunoglobulin isotype switching, and selective gene activation/expression during ontogeny.
4. Mechanisms of antigen processing and formation of immunogenic peptide conformers with appropriate Class I and Class II histocompatibility molecules.
5. Mechanisms controlling the distribution, frequencies, differentiation pathways and destinies of hematopoietic progenitor cells.
6. Analyses of events that control the genetic rearrangements, selective gene usage, and both positive and negative selection that affect immunopotent cells in the thymus and/or in the bone marrow.

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Japanese Immunology Board

BROAD GOALS

In 1981, the Immunology Board was formed by the Joint Committee of the U.S.-Japan Cooperative Medical Science Program to study and elucidate cellular and molecular mechanisms of the immune response and to define effective means to control the immune system.

To achieve these objectives several areas of primary importance in immunological research which were to be carried out by the Board members were identified. They were:

1. Molecular studies of the genetic organization of the lymphoid system.
2. Cellular and molecular studies of lymphocyte activation, proliferation and differentiation.
3. Immunogenetic approaches to the study of lymphocyte interactions, with emphasis on the role of histocompatibility gene products in the regulation of the immune response.
4. Cellular and molecular studies of the regulation of the immune response, with emphasis on diagnosis, therapy, and prevention of diseases involving disordered immune function or affected by the immune response.
5. Application of techniques for the development of stable cell lines and of somatic cell hybrids to the study of the regulation of lymphocyte activation and function and to the preparation of monoclonal antibodies, lymphocyte receptors, and both specific and non-specific lymphocyte products.
6. Mechanisms to support the interaction of immunologists and the exchange of information and materials between the two countries.

PROGRESS IN PRIORITY AREAS

For the past 5 years following the initial period of 3 years, considerable progress has been made by members of Japanese Immunology Board in some of these areas such as molecular and functional studies on IL-6 and IL-2, structure and functions of human MHC (HLA) genes and molecules and mechanisms of regulation of immune system.

Studies on the Activation, Proliferation, and Differentiation of Lymphocytes.

Discovery of interleukin IL-6, molecular cloning of the IL-6 receptor and immunobiological significance of the IL-6.

Extensive studies have been carried out after the discovery of IL-6 as the B cell differentiation factor to elucidate its biological functions and the possible role(s) in various diseases. The cDNA for IL-6 was cloned and studies with recombinant IL-6 demonstrated a wide variety of its functions against different cells and tissues. The function of IL-6 includes: induction of antibody production of B cell differentiation factor. Indication of cytotoxic T cells (CTL), activation of hematopoietic stem cell, induction of differentiation of myeloid leukemic cells into macrophages, indication of rat pheochromocytoma cells into nerve cells and induction of major acute phase proteins as a hepatocyte stimulating factor.

To elucidate the mechanisms of multiple functions of IL-6, the cDNA coding for IL-6 receptor was cloned and its primary structure was determined. The amino acid sequence of IL-6 receptor revealed that the receptor belongs to the immunoglobulin gene superfamily. Intracytoplasmic portion of the receptor does not possess any unique structure for signal transduction and binding of IL-6 to its receptor triggers association of the second polypeptide (gp130) that may be involved in the signal transduction.

It was of particular interests that IL-6 promotes the growth of myeloma/plasmacytoma cells. Myeoloma cells freshly isolated from bone marrows of such patients can utilize their own IL-6 for their growth. Thus, IL-6 appears to be an autocrine growth factor for human myeloma cells, suggesting the possibility that deregulated expression of IL-6 in B lineage cells might be involved in oncogenesis of multiple myelomas.

Studies on Physiological Functions of IL-2 by Using Transgenic Mice.

Two strains of transgenic mice which carry the cDNA of either human IL-2 or the L chain of its receptor under the control of H-2K^d promotor were constructed. The IL-2 transgenic mice expressed human IL-2 mRNA in thymus, spleen, bone marrow, lung, muscle, and skin and IL-2 protein in their sera. The IL-2 transgenic mice suffered from alopecia and pneumonia and the immune responsiveness of their spleen cells to foreign antigens was markedly impaired, whereas their spleen cells responded well to polyclonal lymphocyte activators suggesting that constitutive expression of human IL-2 might have affected the repertoire formation of T cells in these mice. The IL-2 receptor (IL-2R) transgenic mice were apparently healthy.

Crossing of IL-2 and IL-2R transgenic mice produced hybrid mice expressing both the ligand and the receptor constitutively. These hybrid mice had an unusual expansion of thy-1 + /$CD3^-$ 4^-8^- large granular lymphocytes with the elevated NK activity. The increase in number and activity of NK cells seems to be responsible for both the severe interstitial pneumonia and lymphocyte depletion in the spleen. It was also found that selective loss of Purkinje cells in the cerebellum seen in these mice gave rise to their disturbed gait. All the transgenic mice died by 4 weeks of age.

Studies of Human Major Histocompatibility Complex (MHC)

Structure and evolution of HLA-B5 crossreacting group (CREG).

Overall comparison of 40 HLA class I sequences has predicted that the diversity of HLA specificities was formed by amino acid substitutions, which were generated more frequently by recombination events between alleles of the same locus than those between alleles of different loci. HLA-B5 CREG includes B51, Bw52, Bw53 and B35 were suspected to have a limited polymorphism and to have evolved from a common ancestor.

Genes coding for these B5 CREG specificities were cloned and their primary structures were determined. Comparative studies on the amino acid sequences of B51 and Bw52 revealed that the sequence of HLA-B51 can be derived by the simple combination of a point mutation and an exchange event between Bw52 and B8.

HLA-B35 was shown to be identical to Bw58, except in the alpha 1 domain. The alpha 1 domain of B35, except Bw4/Bw6 public epitopes-associated sequences, was identical to that of B51 that was hypothesized as an intermediate gene between B51 and Bw52. These data suggest that B35 has evolved from Bw58 by two steps: an *in vivo* replacement of the alpha l domain with B51 and genetic exchange of Bw4 with Bw6 gene.

The sequence of HLA-Bw53 demonstrated that Bw53 and B35 differed only by the amino acids associated with the public Bw4/Bw6 epitopes. Thus, the sequence of Bw53 indicated that Bw53 is evolutionally located between Bw58 and B35. Taken together studies on the B5 CREG genes and molecules demonstrated that the diversity of these HLA class I molecules is formed by recombinational events in and around the exon 2 of the genes. It should also be noted that exchange of public sequences (Bw4/BwG) creates new private specificities.

Specificities of these B5 CREG molecules were originally identified by alloantisera. Cytotoxic T lymphocyte (CTL) clones discriminating these specificities were generated in repeated *in vitro* stimulation and subsequent cloning in the presence of rIL2. These results demonstrated that T cells can also discriminate these closely related specificities at their clone level.

Structure and expression of HLA-C "blank" genes

Two genes (Cb-1 and Cb-2) encoding HLA-C blank antigens, which were not detectable by alloantisera, were cloned and their primary structures were determined and compared with those of known C-locus genes. The similarity of amino acid sequence between Cb-1 and Cw1 was the highest: Five amino acid substitutions between these molecules were located on the Beta strands of alpha 1 and alpha 2 domains, suggesting that they might change the conformational determinants on the alpha helical region of Cw1.

Cb-2 was the closest to Cw2.2. Six of nine amino acid substitutions between these molecules were found on alpha 1 and alpha 2 domains, where as three remaining substitutions were scattered on the leader peptide, alpha 3 domain and the transmembrane. Two substitutions (residues 73 and 163) of the alpha helical region of alpha 1 and alpha 2 domains and one (residue 16) on exposed loop may make a new allodeterminant. Both gene products were stably expressed on the transfectants. These results suggested that the HLA-C blank antigens are most probably expressed on cells in C blank individuals but incapable of generating alloantibodies.

Studies on Human Minor Histocompatibility Antigens. It has been suspected for many years that rejection of some HLA identical organ grafts and GvH reaction following HLA identical bone marrow grafts are due to disparity of human minor histocompatibility antigens (hmHAs). Thus, the biological importance of hmHAs in the fields of clinical transplantation has been well recognized.

Generation of CTL clones against human minor histocompatibility antigens (hmHAs)

CTL clones were generated from PBLs of a patient who had been grafted with the kidneys from his mother and two HLA identical sisters by repeated *in vitro* stimulation in the presence of r-IL2. Of 8 CTL clones produced, 4 clones recognized an hmHA (hmHA-1) expressed on the cells of mother and an HLA identical sister (2nd donor), two other clones recognized another antigen (hmHA-2) on the cells of father and another HLA identical sister (3rd donor), and the remaining 2 clones still another antigen (hmHA-3) on the cells of father and 2nd donor.

Linkage of the hmHAs under investigation to HLA was excluded by the fact that these CTL clones were generated in response to the genotypically HLA identical cells and all these antigens segregated independently from HLA in the family. Studies of the hmHAs on panel cells indicated that polymorphism of the hmHAs is very limited.

Presentation of hmHAs by HLA class I molecules to allospecific CTLs

Studies of panel cells with known HLA phenotypes revealed that CTL recognition of hmHA-1 was restricted by HLA-B35 and those of hmHA-2 and hmHA-3 were restricted by HLA-B38. The CTL clones under investigation selected HLA-B35 and B38 rather than other possible candidates, notably HLA-A2. This would indicate that restriction molecules for hmHA recognition are not necessarily limited to particular HLA specificities such as HLA-A2 and B7 as previously claimed.

The HLA-B35 restriction of the hmHA-1 specific clones was further substantiated by the fact that these CTL clones killed HLA-B35 transfected Hmy2C1R cells, but not HLA-B51, Bw52 or Bw53 transfected Hmy2C1R cells. These data demonstrated that the five amino acid substitutions on the alpha 1 domain between HLA-B35 and Bw53, which were known to be associated with the public Bw4/Bw6 epitopes, play a crucial role in the binding of hmHA-1 to B35 molecules. The fact that the hmHA-1 specific CTLs failed to kill Hmy2C1R cells transfected with HLA-B35/B51 chimeric molecules composed of the alpha 1 domain of B35 and others of B51 indicated that 8 residues on the alpha 2 domain also affect the interaction of hmHA-1 and the HLA-B35 molecules.

Immunogenetic Studies on Regulation of the Immune System

Genetic control of immune response in humans.

Family studies and population analyses on immune response to the streptococcal cell well antigen demonstrated that low responsiveness to the antigen was inherited as an HLA-linked dominant trait and its effect(s) was exerted via induction of CD8+ suppressor T cells. The gene responsible for the suppression was designated as an immune suppression gene. This gene was shown to be in a strong linkage disequilibrium with particular alleles of HLA-DR and DQ loci.

The association between low responsiveness mediated by CD8+ suppressor T cells and particular HLA-DR and DQ alleles was also observed on the immune response to schistosomal antigen, *M. leprae* antigen, tetanus toxoid, cryptomeria pollen antigen and hepatitis B virus surface antigen. These results suggest that low responsiveness to these antigens was also controlled by immune suppression genes. Anti-HLA-DR monoclonal antibodies (MoAbs) but not anti-DQ-MoAbs inhibited the immune responses of high responders to these antigens *in vitro*. On the other hand, anti-HLA-DQ MoAbs restored the immune responses of low responders to these antigens. Therefore, it is suggested that under the experimental conditions HLA-DR upregulates and HLA-DQ down regulates the immune responses to the antigens. Thus, the immune suppression gene under investigation may well be a particular allele(s) of the HLA-DQ loculs and HLA-DQ might be epistatic to HLA-DA in the regulation of immune responses in man.

Biochemical and functional characterization of I-J polypeptide with reference to immunosuppression.

I-J has been an enigmatic molecule on T cells which undergoes a systematic somatic alteration according to the environmental MHC during ontogeny of the T cells as demonstrated in radiation bone marrow chimeras and class II gene transgenic mice. Thus, I-J is not a direct product of an MHC gene as originally thought, but is an adaptive molecule to a self MHC polymorphism, and it is most likely a receptor-like molecule for self. To study the molecular nature of I-J, a series of IL-2 dependent T cell clones from different haplotype origins including radiation bone marrow chimeras were established. They were either helper (Th) or suppressor (Ts) clones with different MHC restriction specificities and mostly CD4+ Th and Ts clones, except for a few CD8+ Ts clones. Ts subtype was defined by its inability to produce both IL-2 and IL-4, lack of helper activity for B cells, and by its strong inhibitory activity for the antibody response of MHC-matched T and B cells.

Many of the H-2^k-restricted clones were found to be positive for the staining with MoAb to I-J^k epitope. Both I-Ah and I-E^k-restricted clones derived from different origins, including H-2^b, H-2^k chimeras expressed the same IJ^k epitope. The I-J molecule was not co-modulated with TcR/CD3 complex.

By immunoprecipitation and subsequent one- or two-dimensional gel analyses of the lysate of these T cell clones, I-J molecule was found to be a novel dimeric surface molecule of MW 86,000 composed of 43,000 glycopeptide subunits differing from TcR heterodimer, MHC class II antigen, and previously known dimeric proteins of T cells, A monomeric form also exists on some T cell clones. Protein sequencing and Gene cloning approaches are now underway.

The I-J polypeptide seems to play an important role in the regulation of early signal transduction in T cells after antigen-recognition. When T cell clones with the I-J^k epitope were pretreated with the MoAb to I-J^k, the Ca^{++} influx induced by a subsequent stimulation with antigen-pulsed antigen-presenting cell was greatly inhibited. The Ca^{++} response induced by anti-TcR heterodimer but by anti-CD3 or Con A was similarly inhibited by the anti-I-J. The ligation of I-J molecules by intact antibody but not by Fab was required for the initiation of suppression. The inhibition pattern of Ca^{++} influx was very similar to that observed in Th clones preincubated with Ts clones. These results indicate that the negative signal from I-J receptor may inhibit the expansion of self MHC-reactive T cells both in ontogeny and in periphery.

SIGNIFICANT ACCOMPLISHMENTS

The Biology of Interleukin 6

Interleukin 6 (IL-6) was originally identified as a T cell derived lymphokine, which induced the final differentiation of B cell differentiation factor (BCDFP or B cell stimulatory factor 2 (BSF2). The cDNA for IL-6 was cloned in 1986. Studies done with recombinant IL-6 demonstrated that the function of IL-6 was not restricted to B lineage cells but showed a wide variety of biological functions on various tissues and cells i) induction of antibody production in B cells as B cell differentiation factor, ii) induction of cytotoxic T cells as cytotoxic T cell differentiation factor, iii) activation of hematopoietic stem cells as multicolony stimulating factor, iv) induction of differentiation of myeloid leukemia cells into macrophages, V) induction of differentiation of rat pheochromocytoma cells (PC12) into nerve cells and vi) induction of major acute phase proteins as hempatocyte stimulating factor. Since antibody response, hematopoiesis, and acute phase reaction are three major responses against infection, inflammation and tissue injuries, the results suggest that IL-6 may play a central role in host defense mechanisms.

One of the interesting activities of IL-6 is the growth stimulation of myeloma/plasmacytoma cells. The studies with myeloma cells freshly isolated from bone marrows of myeloma patients demonstrated that IL-6 was an autocrine growth factor for human myeloma cells, and suggested that the deregulated expression of IL-6 in B lineage cells might be responsible for oncogenesis of human multiple myelomas. This was further confirmed by the preparation of transgenic mice using human IL-6 gene conjugated with the immunoglobulin enhancer (Eu).

IL-6 provides multiple signals through IL-6 receptors, growth stimulation, growth inhibition and induction of differentiation or the specific gene expression. In order to know the molecular mechanisms how one cytokine provides such multiple signals, the molecular structure of the receptor should be revealed. The cDNA encoding the IL-6 receptor was also cloned by our group in 1988. The amino acid sequence of IL-6 receptor demonstrated that the receptor belonged to the immunoglobulin gene superfamily. Intracytoplasmic portion of the receptor does not have any unique structure for signal transduction, such as tyrosine kinase domain. Interestingly, binding of IL-6 with IL-6 receptor triggered the association of the second polypeptide chain (gp130), which may be involved for the signal transduction.

As summarized here, essential parts of the studies on IL-6 have been: discovery of IL-6 as B cell differentiation factor; molecular cloning of the IL-6 receptor; and the relationship between IL-6 and various diseases.

Studies on Physiological Functions of Interleukin-2 by using Transgenic Mice

We constructed two strains of transgenic mice that carry the cDNA of either human interleukin-2(IL-2) or the L chain of its receptor under the control of the H-2K^d promoter. The IL-2 transgenic mice expressed human IL-2mRNA in the thymus, spleen, bone marrow, lung, muscle, and skin. Human IL-2 protein was also detected in their sera. The IL-2 transgenic mice suffered from alopecia and pneumonia, but no typical autoimmune reactions seemed to be involved in these lesions. In the epidermis of the IL-2 transgenic mice there was an increase in Thy-1^{+} dendritic epidermal cells (Thy-1^{+}DEC), which might be responsible for the skin lesion of the mice. Immune responses of their spleen cells against antigens were significantly impaired whereas the spleen cells responded well to polyclonal lymphocyte activators. This suggests constitutive expression of IL-2 might have affected the repertoire formation of T cells, resulting in immune dysfunctions in the transgenic mice. The IL-2 receptor (IL-2R) transgenic mice were healthy probably because the transgene-encoded L chain of human IL-2R can hardly bind endogenous murine IL-2. We crossed the IL-2 and IL-2R transgenic mice to yield the hybrid mice expressing both the ligand and the receptor constitutively. The hybrid mice showed severe growth retardation and their movement was highly ataxic. All the hybrid mice died by 4 weeks of age. Histological examination revealed the selective loss of Purkinje cells in the cerebellum of the hybrid mice, which seemed to be the primary cause of their disturbed gait. In addition, we found severe interstitial pneumonia and lymphocyte depletions in the thymus and spleen of the hybrid mice. In the spleen of the hybrid mice, we observed an unusual expansion of Thy-1^{+}/CD3^{-}4^{-}8^{-} cells. They had the morphology of large granular lymphocytes (LGL) and bore the elevated natural killer (NK) activity. The T-cell receptor genes of the LGL were in unrearranged configurations. Taking these facts together, we concluded that the abnormally expanded LGL in the hybrid mice were classical NK cells of non-T-cell lineage. The majority of infiltrating lymphocytes in the lung of the hybrid mice were also shown to be the expanded NK cells. Surprisingly, the hybrid mice had no expansion of T cells. This may reflect the different signal-transducing pathways of the IL-2/IL-2R system in T cells and NK cells. The hybrid mice showed neither typical autoimmune reactions nor T cell malignancies.

FUTURE GOALS

Biological significance of interleukins especially IL-2, IL-4, IL-5 and IL-6 in various diseases such as autoimmune diseases and malignancies will be investigated. Genes and molecules that regulate the immune response in humans will be defined by using recombinant technology as well as transgenic mice and/or Scid/Hu mice. T cell recognition in the context of MHC restriction will be delineated by using human minor histocompatibility antigens as a possible candidate for "allo-peptide."

PROJECTS SUPPORTED BY THE PROGRAM

Investigator and Institution	*Title*
Tasuku Honjo Kyoto University, Kyoto	Organization and rearrangement of immunoglobulin genes. Molecular cloning and expression of cytokine genes.
Kyoichi Kano University of Tokyo	Immunological studies on autoimmune diseases. Structural analysis of human minor histocompatibility antigens.
Tadamitsu Kishimoto Osaka University, Osaka	Cellular and molecular studies of B cell growth and differentiation. Molecular analysis of biological functions of interleukin 6.
Takehiko Saszuki Kyushu University, Fukuoka	Analysis of HLA system in the regulation of human immune response.
Tomio Tada University of Tokyo, Tokyo	Studies on the circuits of immune suppression. Mechanisms of immune suppression.

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Joint Scientific Conferences

IMMUNOLOGY BOARDS

Date: July 20–23, 1986

Place: Toyama City, Japan

Summary: The fifth meeting of the Boards featured sessions concerned with (a) ontogeny, activation and differentiation of B lymphocytes, (b) regulation of T cell responses, and (c) autoimmunity and tumor immunology. There were 19 scientific lectures presented by 11 speakers from Japan and 8 speakers from the United States. The meeting was well attended, more than 100 persons from Japan and the United States were in the audience.

Date: December 1–4, 1987

Place: Monterey, California

Summary: The highlight of this meeting was a 1-day joint scientific program with the U.S. Japan Virology Panel. The title of that program was "Immunopathology of Virus Infections" and featured four speakers each from Japan and the United States. In addition, 2 days were devoted to a separate meeting of the Board. The scientific presentations were organized around four topics: (a) ontogeny and differentiation of the immune system, (b) immunoregulatory mechanisms, factors and molecules, (c) genetic controls and molecular approaches to immune system dysfunctions, and (d) immunologic dysfunctions and disease. Many postdoctoral fellows and junior faculty from the western United States attended the meeting.

Date: December 1–3, 1988

Place: Kyoto, Japan

Summary: This was an outstanding scientific meeting that included top immunologists from the United States and Japan as speakers. In addition to scientific lectures from each of the United States and Japanese Board Members, there were 6 lectures by invited speakers from the United States and 8 lectures by invited participants from Japan. The presentations were organized around the following topics: (a) gene regulation, (b) development, (c) MHC and surface antigens, (d) T cell receptors, and (e) transgenic and SCID mouse. There was a large audience (in excess of 100) and the discussions were lively and productive.

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Board of Directors
Sasakawa Medical Health Foundation

Profiles

U.S. LEPROSY PANEL

Dr. Patrick J. Brennan

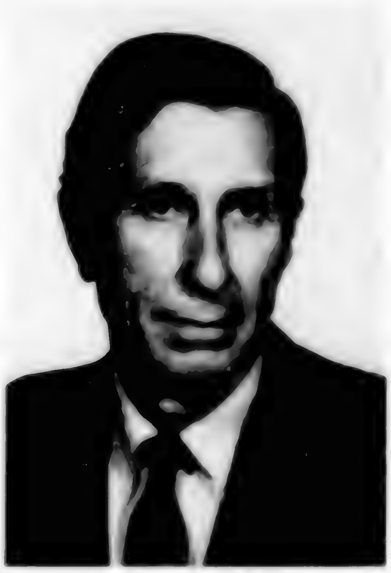
Chairman (1986–)

Dr. Brennan received his Ph.D. degree from Trinity College, Dublin, Ireland in 1965. He has been actively engaged in research on the composition and metabolism of mycobacteria for many years and has been investigating the structure of type specific antigens and lipids of various mycobacterial species. Dr. Brennan routinely purifies leprosy bacilli from infected armadillo tissues and has isolated and characterized *Mycobacterium leprae* lipid antigens from purified cells and infected tissue. He is on the Editorial Board of the Journal of Bacteriology and has participated in many Joint U.S.-Japan Leprosy-Tuberculosis Conferences. Presently Dr. Brennan is a professor in the Department of Microbiology, Colorado State University. He has been chairman of the U.S. Leprosy Panel since 1986.

Dr. Robert Hastings

Chairman (1981–1985)

Dr. Hastings received his M.D. in 1962 from the University of Tennessee, College of Medicine, Memphis, and his Ph.D. in Pharmacology from Tulane University School of Medicine, in 1971. He has served on the U.S. Leprosy Panel as a member from 1977–1981, and as chairman from 1981–1986. Since 1964, he has been Chief, Laboratory Research Branch, and Chief, Pharmacology Research Department at the Gillis W. Long Hansen's Disease Center in Carville, LA. He was editor of the International Journal of Leprosy and served as a consultant for WHO an other organizations. Dr. Hastings is responsible for the manufacture, encapsulation, quality control, distribution and reporting on leprosy patients receiving the drug thalidomide in the U.S. and Canada. He has held appointments at the Tulane Medical Center and the Louisiana State University. Dr. Hastings has published extensively and edited the textbook *Leprosy*. His major research interests are thalidomide, leprosy, immunopharmacology, and chemotherapy of infectious diseases.



Dr. Charles C. Shepard
Chairman (1965–1977)

Dr. Shepard was Chief of the Leprosy Section at the Centers for Disease Control (CDC) for more than 30 years and was in the Public Health Service for 44 years including working at the NIH from 1941 to 1948, the Biochemistry Institute, Uppsala, Sweden, 1948 to 1949 and the NIH Rocky Mountain Laboratory from 1951 through 1953. He joined CDC in 1953 and early on was primarily involved in research on rickettsial diseases. Prior to 1981, he was responsible for both leprosy and rickettsial disease research at the CDC, but after that was totally involved in work on leprosy. He is perhaps best known for his discovery of the ability of the leprosy bacillus, which had evaded all previous attempts at cultivation in the laboratory, to grow in the cooler tissues of the footpads of mice. This was a singularly important discovery which has greatly advanced research on the chemotherapy and immunology of leprosy. Dr. Shepard was chairman of the U.S. Leprosy Panel from 1965 to 1977. Dr. Shepard died in 1985.

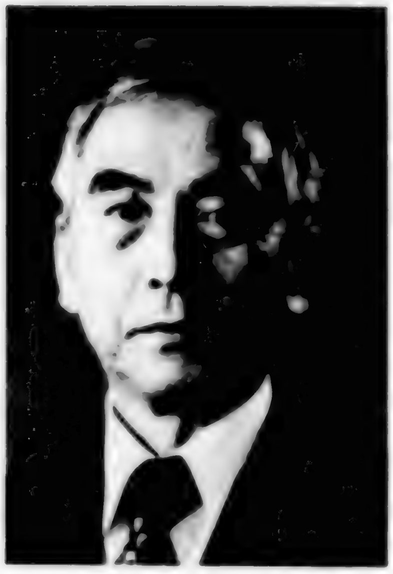
JAPANESE LEPROSY PANEL

Dr. Tatsuo Mori
Chairman (1988–Present)

Since Dr. Mori graduated from the School of Medicine at Osaka University, he has devoted himself to the study of leprosy in the Research Institute for Microbial Diseases, Osaka University. Two years ago he was appointed fifth president of the National Institute for Leprosy Research.

Dr. Mori was famous for the study of biochemical studies in murine leprosy and leprosy bacillus (Sakurane Award), following experimental cultivation of murine leprosy bacillus, inoculation of leprosy bacillus into the nude mouse (Sakurane Award), improvement of the collection method of leprosy bacillus and development of a new infection theory of leprosy all done in the Research Institute for Microbial Diseases. He accomplished many splendid results to contribute to the superb tradition of the Department of Leprology in the Research Institute for Microbial Diseases, Osaka University.

Dr. Mori was appointed the fifth president of the National Institute for Leprosy Research, and the additional post of the fourth Chairman of the Japan-U.S. Cooperative Medical Science Program and Editor of the Japanese Journal of Leprosy. The new research building which was long a desire of all researchers in this Institute was constructed and an increased commitment to accept students from Southeast Asia complimented his becoming Director.



Dr. Masahide Abe

Chairman (1980–1987)

Since Dr. Abe graduated from the School of Medicine at Tokyo University, he devoted his life exclusively to research in serology. He managed the serology laboratory of the National Institute for Leprosy Research from the time of its founding, after leaving the Department of Serology of Tokyo University where he worked with Professor Ogata.

After establishing the method of fluorescent immunoassay for the patients' serum reaction, he applied the technique to the high-risk groups of familial infection including uninfected children, promoting this new prophylactic treatment. Based upon this achievement, he received the Sakurane Prize, a reward from the Japan Leprosy Society. At the same time, he received the Hideo Noguchi Prize for his long-term work on the leprosy serum reaction. Dr. Abe assumed office as the fourth president of the National Institute for Leprosy Research, and in addition, was the Japanese representative of the Experts' Subcommittee of the Japan-U.S. Cooperative Medical Science Program. As such, he carried out this serious and arduous task for a long period of time in concert with Drs. Hastings and Brennan.

Dr. Yoshio Yoshie

Chairman

Dr. Yoshie took the elite step of entering the School of Medicine of Tokyo University after completion of the Third Higher School, before becoming a physician in otolaryngology. He then investigated areas concerning Hansen's disease, became a medical officer in the National Leprosarium Tama Zenshoen, and took office as the second president of the National Institute for Leprosy Research. His subsequent history included his long-term activities, in concert with those of Dr. Shepard, as the Japanese representative of the Leprosy Panel, Experts' Subcommittee of the U.S.-Japan Medical Science Cooperation Program from the time of its founding. Meanwhile, he devoted himself to the cultivation of the causal bacterium for leprosy, finally succeeding in several-fold proliferation of this microorganism. Regrettably, however, there was no method of inoculating animals at that time to confirm the viability of the microorganism. He afterwards received the Damian Datton Prize for his work. Last year, the then-87-year-old doctor presented himself in quite a good state of health, at the celebration of the completion of the new laboratories building at the National Institute for Leprosy Research. The Japanese leprosy research community is happy that Dr. Yoshie still follows the research activities of the leprosy field.

Dr. Masashi Nanba

Chairman

Spiritually based on the principle of equality in human rights and humanity, Dr. Nanba came to admire Dr. Mitsuda, which resulted in his life being wholly dedicated to the studies in the field of Hansen's disease. He has been a pathologist who willingly resigned his life to the treatment of patients with this disease. Dr. Nanba liked drinking, talking to young researchers, and insisting that those who study fundamental medicine should first see patients with leprosy. He became the third president of the National Institute for Leprosy Research, succeeding Dr. Yoshie, and at the same time, worked for a long period as the second Japanese representative of the Leprosy Panel Experts' Subcommittee of Japan-U.S. Cooperative Medical Science Program. At the celebration of the completion of the new laboratory building at the National Institute for Leprosy Research held last year, Dr. Nanba, at the age of 79, appeared to be in good health. He is still active in the field of medicine.

GUIDELINES

LEPROSY PANELS
United States-Japan Cooperative Medical Science Program

All areas of leprosy research are relevant, but the following are of special interest and importance from the present-day perspective:

1. Further definition of the immunogens of the leprosy bacillus; development of recombinant DNA vectors for the production of defined antigens; development of procedures for the purification of recombinant antigens and for the synthesis of their antigenic domains.
2. Identification of individual T- and B-cell epitopes on *M. leprae* antigens; study of the issue of their gentic restriction; explore the question of segregation of epitopes toward T-cell subsets on defined antigens.
3. Characterization of the cell wall components of *M. leprae* with a view to isolation and differentation of suppression-inducing and helper/protection epitopes.
4. Further exploration of the genome and metabolism of *M. leprae* with a view to mapping genes of interest, to isolating genes that control synthesis of complex cell wall glycoconjugates, and to allowing transfer of genes in order to construct, ultimately, through gene manipulation, whole cell or subunit vaccines. By a greater understanding of the metabolism of *M. leprae*, it may be possible to devise an appropriate culture medium and return again to the long-time goal of the growth of *M. leprae*.
5. Development of assays, based on antibody or antigen detection or recognition of nucleic acid fragments, suitable for the diagnosis of paucibacillary leprosy.
6. Continuing expansion of the variety and supplies of monoclonal antibodies, recombinant DNA vectors, T-cell clones, and natural antigens, for the fostering of cooperative research.
7. Continuation of animal model and human studies on the modulation of host response to innovative chemotherapy, natural recombinant antigens, adjuvants, and lymphokines.
8. Continuation of efforts using animal models to explore the efficacy of pure antigens/epitopes in conferring protective immunity.

U.S. Leprosy Panel

BROAD GOALS

Leprosy, or Hansen's Disease, continues to be a major endemic disease in most countries of Asia, Africa, and Latin America. However, it is difficult to estimate the approximate number of cases of leprosy in such areas. Case diagnosis and definition are not always clear or consistent, and the enumeration of cases in many regions is incomplete or irregular. Nevertheless, estimates extrapolated from available data from time to time, for 1966 and 1976 were 10.8 and 10.6 million cases, respectively. Prevalence is currently estimated at 10–12 million cases. Approximately 1.6 billion people live in areas where the estimated prevalence is over 1 case per 1,000 persons, and thus this vast population may be considered at risk.

Information on leprosy cases registered for treatments is much more reliable than information on estimated cases, as it is based on actual records. There has been a steady increase in the number of registered cases over the past 20 years: 2,831,775 for 1966; 3,599,949 for 1976; and 5,368,202 for 1985; the latter figure represents an increase of 49.1% over 1976 and 89.6% over 1966. The prevalence rates of registered cases have correspondingly increased from 0.84 cases per 1,000 population in 1966 to 0.88 in 1976, and 1.2 in 1985. Although only a proportion of estimated cases ever get registered for treatment, the information on registered cases reflects to a large extent the global leprosy situation.

Southeast Asia, of direct concern to the Japanese Leprosy Panel, contributes the largest share of registered cases in the world, and it has shown a dramatic increase over the past two decades, from 790,851 in 1966, to 1,748,468 in 1976, to 3,737,157 in 1985. On the other hand, Africa has shown a steady but modest decline in the number of registered cases over the years, from 1,685,526 in 1966, to 1,398,220 in 1976, to 987,607 in 1985. In the region of the Western Pacific, contiguous to Japan, the figures indicate a slight increase for 1976 (128,325 registered cases) and a marked increase for 1985 (245,753 registered cases).

The Americas, of direct import to workers in the United States, has shown a steady increase in the numbers of registered cases, from 177,813 in 1966 to 241,248 in 1976 to 305,999 in 1985. It is clear that the largest proportion of this increase is contributed by Brazil. The magnitude of the present-day problem of leprosy in Brazil is only now being fully realized by research workers and international agencies. As of the end of 1987, Brazilian Government personnel indicated that there were 239,328 cases in active registration which means a national prevalence rate of 1.7 per 1,000, among the highest in the world; in the States of Acre and Amazonas, rates of 11.2 and 12.9 per 1,000 have been recorded. Also, leprosy is an urban problem in Brazil. For instance, Sao Paulo City has 10,822,614 inhabitants, making it the largest city in South America, and Sao Paulo State has a population of 31,501,514 people. In 1987, leprosy prevalence in Sao Paulo State was 12.6 per 1,000, and the detection of new cases reached 8.95 per 100,000; in Sao Paulo City, the prevalence figure was 8.3 per 1,000 persons and the absolute number of cases was 12,992. Yet all of these figures represent registered cases; the extent of the real problem in the States of Acre and Amazonas, for instance, is unknown.

In a thoughtful analysis of trends in the worldwide occurrence of leprosy, Dr. M. F. Lechat *et al.* (Wld. Hlth. Statist. Quart. 39:129–137, 1986) pointed out that most countries where leprosy is endemic do not have an adequate health information system to record new cases (i.e., incidence) on a continuous basis and rely on prevalence data, in themselves often questionable. Accordingly, "the only data available at present relate to incidence of manifest disease, and we have no idea of the extent of transmission of infection, or of the probability of transition from infection to manifest disease." These authors conclude that "the development of immunological tests for the detection of leprosy infection is of vital importance if our knowledge of the epidemiology of leprosy is to be improved."

At the beginning of this quinquennium and, indeed, earlier in the life of the U.S.-Japan Cooperative Program, the major problems confronting leprosy research workers were defined as follows: (a) the inability to cultivate the causative organism, *Mycobacterium leprae*; (b) an uncertain proportion of strains of *M. leprae* which had developed resistance to one or more of the first line anti-leprosy drugs; (c) the persisting social stigma of the disease; (d) the characteristic peripheral neuropathy of the disease with secondary crippling; and (e) the fact that leprosy occurs predominantly in developing countries, many of them lacking the necessary resources to implement standard protocols for leprosy control.

Since many of these problems relate to social, economic, and public health issues, the leprosy research community in developed countries, including the U.S., Japan and European countries, have tended to focus on research aimed at providing improved methods for leprosy control. Accordingly, in the early years of the life of the U.S.-Japan Cooperative Program in Leprosy, the major goals and guidelines of the U.S. Leprosy Panel were: cultivation or growth of *M. leprae in vitro*; animal transmission and experimental systems for growth of *M. leprae*; evaluation of chemotherapeutic drugs against leprosy; development of chemoprophylaxis and minimal effective blood and tissue levels of drugs; vaccine development; and elucidation of antigens similar to lepromin and development of the mechanism of lepromin reactions.

PROGRESS IN PRIORITY AREAS

When the ultimate history of leprosy shall be written, the past 5 years will surely be marked as the period in which researchers finally solved the age-old nemesis of the noncultivability of *Mycobacterium leprae*, not by cultivation *per se* but by reproducing its proteins by recombinant means, by ma-

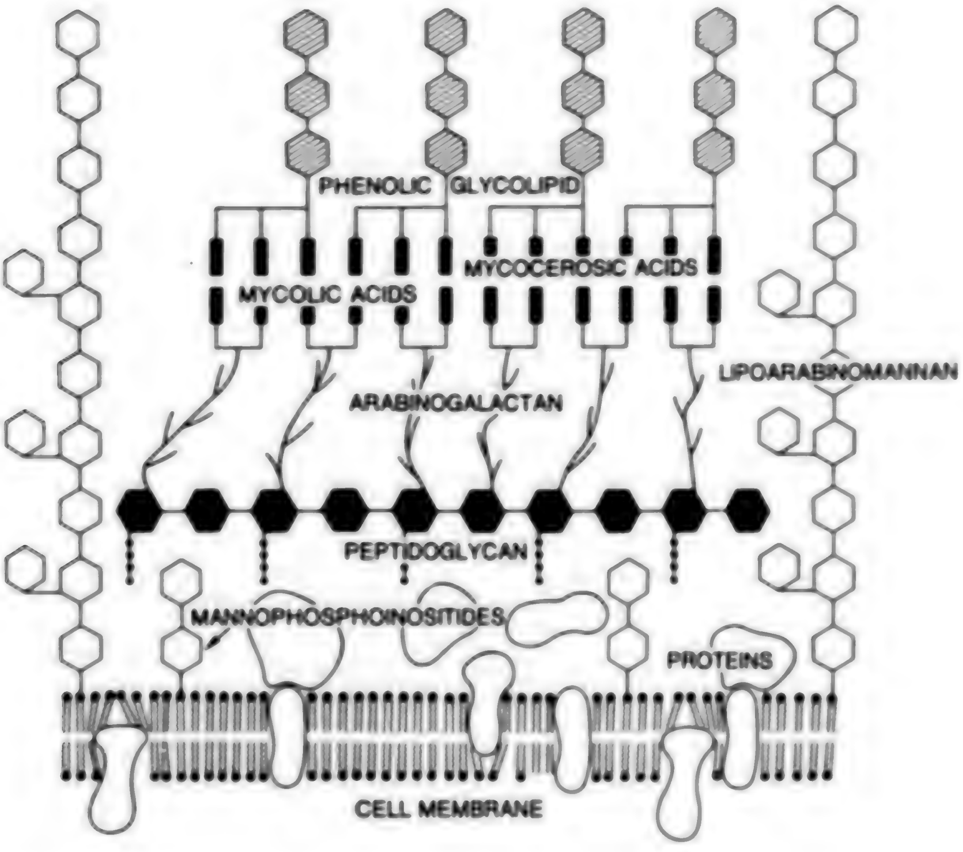


Diagram of the cell wall of *Mycobacterium leprae*.

nipulating its genome and inserting it into cultivable mycobacteria, by elucidating the structure of secondary gene products through use of contemporary chemical tools, and by demonstrating that, although the bacillus would not replicate *in vitro*, it could metabolize, and that this property could be exploited in such a way that true binary fission of the bacillus was not necessary. Thus, the past quinquennium of the U.S.- Japan Joint Leprosy Panels has witnessed truly spectacular progress.

For example, the identification of the genes responsible for the synthesis of most of the immunogenic proteins of *M. leprae*, the cloning of these proteins, the reproduction of large quantities of the recombinant products, and the subcellular location of many of these proteins within the leprosy bacillus have been achieved. Together with these developments has come near spectacular progress in the cloning of T cells of the helper and suppressor phenotype, the recognition that aberrant T cells with the δ heterodimer are preponderant in leprosy lesions, and the use of recombinant proteins and synthetic peptides to map relevant T cell epitopes. Thus, the possibility of identifying the important immunogenic peptides involved in T cell-mediated protective immunity is now at hand, and with it the concept of a subunit vaccine is much more realistic.

Also, the use of recombinant libraries of *M. leprae* and appropriate murine monoclonal antibodies resulted in recognition of several highly conserved, heat-shock proteins in *M. leprae* with intriguing insinuations that some of the immunopathological response in leprosy may be to highly conserved ubiquitous self proteins.

Most of the secondary gene products of *M. leprae* were also fully identified and their role in immunopathogenesis explored. Thus, lipoarabinomannan, by inhibiting activation of macrophages, and phenolic glycolipid I by scavenging oxygen radicals, may abet the ineluctable replication of *M. leprae* within macrophages. Much of the highly immunogenic, massive mycolylarabinogalactan peptidoglycan-protein complex has been defined, and the existence of a true lipid barrier in the cell walls of *M. leprae* regressive toward the permeation of antimetabolites, was recognized. Thus, at last, a rational approach toward the development of anti-leprotics is feasible. This information has led to the formulation of at least a chemical model of the cell wall of *M. leprae* (Figure above).

During the quinquennium, detailed exploration of the *M. leprae* genome was initiated, revealing a universal degree of sequence conservation among isolates from diverse geographical regions and biological sources and also the presence of a unique 470 base pair repetitive sequence. This and other segments of the genome are currently being exploited through the polymerase chain reaction for the sensitive detection of *M. leprae* in biological specimens. Heady progress, unforeseen in the previous quinquennium, has been made in the construction of recombinant, temperate shuttle plasmid vectors for the transfer and the stable expression of *M. leprae* genes in such as BCG vaccine strains. Thus, the dream of a recombinant leprosy vaccine is nearer to reality.

An accomplishment of much significance, not least *vis-a-vis* the older goal of *in vitro* cultivation of *M. leprae*, was the demonstration that the metabolic capability of *M. leprae* can be maintained for long periods and exploited in fundamental and applied ways, even though bacterial mass remains stationary.

The impressive effects of administration of recombinant lymphokines in reconstituting local cell-mediated immunity may open up innovative immunotherapeutic regimens.

While the strength of the U.S.-Japan Cooperative Program in Leprosy is at a fundamental research level, participants in the Program also continue to contribute to the worldwide success of multiple drug chemotherapy in the control of leprosy by developing new regimens, such as minocyline and the fluoroquinolones, and new animal models for the testing of these regimens and the new putative vaccines, both recombinant and native *M. leprae* products.

The start of the quinquennium saw intensive cooperation between U.S. and Japanese participants, particularly in defining the chemical details of the phenolic glycolipids, in synthesizing neoantigens based on the phenolic glycolipid I, and in their implementation in serological assay for the diagnosis of leprosy in the Western Pacific Region. The last few years have again seen a marvelous resurgence in cooperative exercises with joint projects on protein definition and cloning, with the exchange of researchers and with the joint convening and management of conferences.

SIGNIFICANT ACCOMPLISHMENTS

Success in the accomplishment of many of the earlier goals was brought about largely through the intercession of the U.S.-Japan Cooperative Program and other international programs. For instance, the earlier lessons derived from rodent models on the use of multiple drug regimens were extended to vast human populations such that it is now reckoned that over 80% of the leprosy patients of India will be under multiple drug therapy by the turn of the century, and drug resistance is apparently no longer a serious problem. The advent and then the widespread implementation of the mouse footpad model now allows the careful monitoring of drug resistance and the testing of new anti-leprotics. In addition, the availability of the armadillo model of lepromatous leprosy and a facile protocol for *M. leprae* isolation allowed the initiation of first generation candidate vaccine trials involving killed *M. leprae* and live BCG, in Venezuela, Malawi, and recently in India. Also, the provision of unprecedented numbers of *M. leprae* through the armadillo attracted to the field new generations of investigators—biochemists, chemists, microbial geneticists, notably from the United States, Japan, Europe, India, Mexico, Brazil, and India—leading to spectacular developments in the antigenic and chemical definition of the bacillus, in the production of a large slate of murine monoclonal antibodies and in isolation of the genome of *M. leprae* in intact form. A most significant development during this phase of the 25-year history of the Program was the finding of large quantities of a unique glycolipid antigen in *M. leprae* endowed with its own *M. leprae*-specific trisaccharide moiety. A consortium of U.S. and Japanese workers in the early 1980's were able to fully characterize this triglycosyl unit, to chemically synthesize it, and produce unlimited quantities of neoantigens suitable for the diagnosis of lepromatous leprosy and the monitoring of aspects of the disease.

Precise molecular definition of the protein antigens of *M. leprae* began also in the early 1980's with the development of relevant monoclonal antibodies. In a WHO-sponsored, collaborative study involving U.S., Japanese, and European researchers, a panel of *M. leprae*-specific monoclonal antibodies were used in Western blots to delineate six distinct protein antigens in disrupted *M. leprae*. These are generally referred to by their apparent molecular weights, as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoreses, as the 70 kD, 65 kD, 35 kD, 28 kD, 18 kD, and 12 kD proteins. The limited number of protein antigens identified by monoclonal antibodies and the frequency with which the same antigens have been detected by antibodies from different laboratories has led to the suggestion that these are the immunodominant protein antigens of *M. leprae*. The most spectacular use of these antibodies has been to screen the lambda gt11 *M. leprae* recombinant DNA expression library resulting in the isolation of clones expressing the 12, 18, 28, 36, 65, and 70 kD proteins. In addition, efficient recombinant DNA strategies were designed to deduce the amino acid sequences that comprise specific antigen determinants in such proteins. This approach was best used to define antibody epitopes encoded within the gene for the 65 kD protein antigen of *M. leprae*; synthetic peptides and monoclonal antibody inhibition assays were used to the same effect. In all, about 12 antigenic determinants reacting with monoclonal antibodies and approximately 6 which react with human T-cell clones were recognized on the 65 kD polypeptide. The amino acid sequence of the 65 kD protein of *M. leprae* showed 95% homology with its *M. tuberculosis* counterpart, and, of course, it is now recognized that these belong to the family of ubiquitous, highly conserved heat-shock or stress proteins with implications well beyond leprosy. The complete gene of the 18 kD protein was also isolated and sequenced and the protein expressed in *E. coli* and a yeast expression system, and a high proportion of *M. leprae*-specific T-cell clones from donors immunized with a killed *M. leprae* vaccine recognize this protein.

During this period, starting in the early 1980's, with the isolation of intact DNA, the genome of *M. leprae* began to also receive considerable attention. Not only were genes for many of the soluble, immunogenic proteins identified, cloned and expressed, but also so were genes for known metabolic functions. The powerful tool of restriction fragment length polymorphism was used to examine the degree of conservation of nucleotide sequence within the chromosomal DNA from *M. leprae* obtained throughout the biosphere; it emerged that the genome of *M. leprae* is remarkably stable and conserved. In near revolutionary developments, one laboratory reported on the successful development of a variety of shuttle vectors that in the future will allow the cloning of large fragments of DNA into cultivable mycobacteria and the stable integration and expression of such DNA.

Our understanding of the T-cell response in leprosy also received, a considerable boost during the period of the late 1970's and early 1980's. *M. leprae*-reactive CD3$^+$CD4$^+$CD8 proliferative T-cell clones were isolated from healthy, but *M. leprae*-exposed, individuals. These clones reacted with epitopes within the 65 kD heat-shock protein as well as the 35–36 kD and the 18 kD proteins and the recognition of these showed MHC restriction. Thus, polypeptide segments within those or other proteins may be capable of inducing protective immunity in vaccinated or exposed individuals.

A continuing area of research under the Program, almost since its outset, has been the issue of T cell unresponsiveness in lepromatous leprosy and the question of the role of suppressor T cells therein. In the realization that T cells from lepromatous patients fail to produce adequate amounts of lymphokines and fail to be activated, the issue of whether an effective cell-mediated immune response could be generated in lepromatous individuals by the local injection of PPD was examined. In a rather dramatic fashion, a mere 5 u of PPD resulted in a long-lived, cell-mediated immune response, resulting in the destruction of parasitized macrophages and reduction of viable *M. leprae*, following an influx of T cell subsets, monocytes, and Langerhans cells into the site of inoculation. Importantly, it was also demonstrated that injection of recombinant IL-2 and other lymphokines leads to the local emigration of helper T cells and monocytes, pointing to a potential means of tissue immunotherapy.

The basis of the failure of the macrophage to kill *M. leprae* in the lepromatous form of leprosy was also the subject of intense examination during this period. It was established that macrophages heavily laden with *M. leprae* are markedly defective in a variety of afferent and efferent effector functions. Notably, such granuloma macrophages are refractory to gamma-interferon-induced activation, as demonstrated by markedly reduced microbicidal function, cytotoxicity for tumor cells, superoxide production, and Ia antigen presentation. In some of the most convincing evidence implicating a single defined *M. leprae* product in pathogenesis, it was demonstrated that liparabinomannan drastically inhibited gamma-interferon-induced activation of macrophages, and, thus, lipoarabinomannan was implicated as the first virulence factor of the leprosy bacillus.

During this period, the phenolic glycolipid was also implicated in the pathogenesis of *M. leprae*. Specifically, the trisaccharide unit was implicated as a potent scavenger of intracellular reactive oxygen radicals, thereby perhaps directly facilitating the survival of *M. leprae* within the phagosome. In addition, evidence began to accrue that a sizable proportion of the plentitude of T cells of the suppressor phenotype ($CD8^+$) seen in lepromatous lesions, by elegant cytoimmunological procedures, can be induced to suppress antigenic and mitogenic responses in the presence of the terminal disaccharide unit of the phenolic glycolipid. Methodology was also developed to expand T suppressor clones, although the means by which to maintain those indefinitely in culture is still elusive.

During this period, the leprosy lesion itself, rather than circulating cells, became the focus of intense immunological studies; one report, in particular, demonstrated that 1 in 50 T cells in tuberculoid lesions reacted to the antigens of *M. leprae* compared to a mere 1 in 4,500 in blood. Human lesion-derived T helper ($CD4^+$) clones in conjunction with suitably applied polyacrylamide gel electrophoresis emerged as powerful tools for the identification of the key protein antigen implicated in T helper-induced immunity. Disappointingly, such $CD4^+$ clones do not respond to the available recombinant proteins. However, they responded avidly to the complex, large molecular mass cell wall protein, the so-called CW-P which emerged as one of the most powerful immunogens in the leprosy bacillus.

Important progress was also made in identifying immunoregulatory subsets of CD4 T cells in leprosy lesions. An enrichment of T helper ($CD4^+4B4^+$) was observed in tuberculoid lesions which were in close proximity to macrophages within the granuloma, perhaps facilitating bacterial clearance. In contrast, T suppressor inducer cells ($CD4^+2H4^+$) were found at the periphery of the granuloma, admixed with $CD8^+$ cells, where they may contribute to localization of the cell mediated immune response from causing extensive tissue destruction.

FUTURE GOALS

M. leprae and Its Antigens.

The *in vitro* cultivation of *M. leprae* should no longer be a major goal of the Program.

Since the United States is the source of the infected armadillo colonies, still the world's only source of first generation vaccine and *M. leprae* for experimental purposes, careful attention should be devoted to the upkeep of these facilites and their preservation from other infectious agents, notably other mycobacteria. Mechanisms should be developed to provide larger quantities of pure *M. leprae* to Japanese researchers.

Already the major protein immunogens of *M. leprae* have been identified, cloned and some are available in sizable quantities. The development of large-scale production capabilities for the major protein antigens of *M. leprae* and defined epitopes, by developing over-expression recombinant DNA vectors, should be a priority of U.S. and Japanese workers. Isolation of the native somatic proteins, for comparison with recombinant products, especially in search of *M. leprae*-specific, nonprotein post-translational modifications should be pursued. Particular attention should be devoted by U.S. and Japanese researchers to the issues of adequate purification of native and recombinant proteins and synthesis and purification of peptide epitopes.

Establish the amino acid or genomic sequence for all of the major proteins of *M. leprae* and explore the immunological consequences of N-terminal blockage. Define and synthesize the major T and B cell eptiopes on the major mycobacterial antigens in search of diagnostic agents highly specific and sensitive in serological and skin test assays. Initiate experiments in search of the key epitopes recognized by the T cell receptors. Study the relative immunodominance and genetic restriction of individual epitopes.

Compare the sequence homology of all isolated proteins to known proteins in order to provide clues on physiological function and role in disease pathogenesis.

Pursue the chemical and immunological study of the secondary gene products of *M. leprae*; i.e., the lipids, lipoarabinomannan, the vast mycolylarabinogalactan-peptidoglycan-protein complex with a view to exploring roles in immunity, bacterial persistence, the pathogenesis of reactional states, and in explaining the impermeability of *M. leprae* to many antibiotics.

The Genome of *M. leprae*.

Sequencing of the genome of *M. leprae* should not be a goal in itself.

Instead, isolate and study genes that encode the synthesis of the major protein antigens and also those that control the synthesis of the major cell wall components. Identify and isolate gene expression signals for use in manipulating gene expression.

Continue to develop shuttle vectors in order to introduce *M. leprae* DNA into cultivable mycobacteria. Continue to investigate molecular mechanisms necessary for stable gene introduction and expression.

Continue to explore the nature of the unique repetitive sequence in the *M. leprae* genome with a view to its function and its exploitation.

Expand the use of the polymerase chain reaction technology for the sensitive and specific detection of *M. leprae* infection.

Dissection of the Immune Response in Leprosy.

Examine the mechanism of phagocytosis of *M. leprae* and the chemical and biological basis of *M. leprae* persistence in macrophages, especially in the context of the new surge of knowledge on the composition of *M. leprae*.

Expand studies on processing and presentation of antigen and interaction with α, and, δ T cell receptors. Expand the array of available T cell immunological correlates of protective immunity.

Continue research on T cell subset populations in leprosy lesions. Investigate the functional segregation of epitopes toward T cell subsets of helper *versus* suppressor phenotypes.

Expand research on the immunopathological basis of nerve damage, especially the role of the insoluble fractions of *M. leprae* in immune complex deposition and the effects thereof.

Promote research on the development of innovative experimental models applicable to assays for protective immunity.

Chemotherapy: Human Vaccine Trials.

In view of the large-scale implementation of multiple drug therapy, but still with the possibility of drug resistance emerging years from now, the search for new drugs is to be encouraged. Major responsibility for this activity has traditionally rested with the THELEP component of WHO. However, those efforts can be supplemented by trials testing the efficacy of the various fluoroquinolones, cyclines (especially minocycline), and macrolides. For historical reasons and in view of the excellent animal facilities in some laboratories, chemotherapeutic trials in experimental animal models should continue in the United States and Japan.

Ongoing clinical trials with "first generation" *M. leprae* vaccine (irradiated *M. leprae* and live BCG) are also within the purview of the IMMLEP component of the WHO. In addition, the independently conducted vaccine trials in India, using cultivable mycobacteria, are also being dovetailed into the WHO trials. These activities should be aided directly and indirectly by participants of the U.S.-Japan Cooperative Program by providing *M. leprae* from the armadillo source and other necessary expertise and personnel. In addition, several "subunits" of *M. leprae* are now emerging as powerful immunogens, such as the cell-wall peptidoglycan protein complex, the 35 kD membranous complex, and several individual native proteins, which should be thoroughly tested in the available animal models with a view to large-scale human trials.

PROJECTS SUPPORTED BY THE PROGRAM

GRANTS

Investigator and Institution	*Title*
Joyce A. Benjamins Wayne State University	Autoimmune Mechanisms in the Neuropathology of Leprosy
Barry R. Bloom Yeshiva University	Genetics of Resistance to Leprosy and Leishmaniasis
Barry R. Bloom Yeshiva University	Immunologic Studies in Leprosy
Barry R. Bloom Yeshiva University	Molecular Biology and Immunology of *M. leprae*
Barry R. Bloom Yeshiva University	Mycobacterial Genes, Antigens, and Vaccines
Patrick J. Brennan Colorado State University	*Mycobacterium leprae* Glycolipid Antigens
Patrick J. Brennan Colorado State University	Surface Antigens of *M. leprae* and other Mycobacteria

Thomas M. Buchanan US PHS Gillis W. Long Hansen's Dis. Center	Structure and Antigenicity of Relevant *M. leprae* Proteins
Josephine E. Clark-Curtiss Washington University	Induction of Immunity to *Mycobacterium leprae*
Josephine E. Clark-Curtiss Washington University	*Mycobacterium leprae* Taxonomy and Genetics
Zanvil A. Cohn Rockefeller University	Pathobiology of Human Leprosy
Arvind M. Dhople Florida Institute of Technology	Application of ATP Assays to Patient Care in Leprosy
Arvind M. Dhople Florida Institute of Technology	*In Vitro* Cultivation of *Mycobacterium leprae*
James T. Douglas University of Hawaii at Manoa	The Seroepidemiology of Leprosy
Scott G. Franzblau US PHS Gillis W. Long Hansen's Dis. Center	Metabolic Maintenance and Growth of *Mycobacterium leprae*
Thomas P. Gillis US PHS Gillis W. Long Hansen's Dis. Center	Characterization of T Cell Clones to *M. leprae*
Bobby J. Gormus Tulane University of Louisiana	Characterization of Experimental Leprosy
Bobby J. Gormus Tulane University of Louisiana	Experimental Leprosy/Vaccine Studies in Mangabeys
Robert C. Hastings US PHS Gillis W. Long Hansen's Dis. Center	Nude Mice in Leprosy Research
Timothy J. Holzer University of Illinois at Chicago	Antimycobacterial Activity of Phagocytic Cells
Marcus A. Horwitz University of California Los Angeles	Biology of *Mycobacterium leprae* Monocyte Interaction
Robert C. Humphres SRI International	Induction of Immunity and Tolerance to *M. leprae*
Gilla Kaplan Rockefeller University	Pathobiology of Human Leprosy
James L. Krahenbuhl US PHS Gillis W. Long Hansen's Dis. Center	Immunology and Immunotherapy of Leprosy

Maurice J. Lefford Wayne State University	Delayed Hypersensitivity in Leprosy
Maurice J. Lefford Wayne State University	Limiting Dilution Analysis of *M. leprae* Reactive T-Lymph
Myron A. Leon Wayne State University	Analysis of *M. leprae* Antigen in the Urine
Robert L. Modlin University of Southern California	Function of Cloned Lesion-Derived Lymphocytes in Leprosy
Robert L. Modlin University of Southern California	Immunoregulation in Leprosy Granulomas
Robert L. Modlin University of Southern California	Immunoregulatory T-Lymphocytes in Leprosy Lesions
Nahid Mohagheghpour Medical Research Institute of San Francisco	Immunologic Defects in Lepromatous Leprosy
John H. Peters SRI International	Induction of Immunity and Tolerance to *M. leprae*
Larry S. Schlesinger University of California Los Angeles	Human Monocyte Interactions with *M. leprae*
Richard W. Truman US PHS Gillis W. Long Hansen's Dis. Center	Origin and Risk of Armadillo Leprosy

CONTRACTS

Patrick J. Brennan Colorado State University	Antigens from *M. leprae* obtained from Armadillo Tissue
Robert C. Hastings National Hansen's Disease Center	Maintenance of an Armadillo Colony Infected with *M. leprae*
Eleanor E. Storrs Florida Institute of Technology	Maintain Armadillo Colony Infected with *M. leprae*

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Japanese Leprosy Panel

PROGRESS IN PRIORITY AREAS

Since rifampicin has shown a dramatic therapeutic effect on the lepromatous leprosy patient, multidrug therapy was devised for treating leprosy. Enormous contributions supported cost-free administration of anti-leprosy drugs in Southeast Asia. Results of the trial will be estimated at the end of the century. When drug administration is stopped, for cure, according to negative tests for leprosy bacilli, relapse may occur someday if some leprosy bacilli without contact to drugs live inside the nerve. And, also, a resistant bacillus may arise. Therefore, development of new drugs and a leprosy vaccine is urgent.

Genetic analysis of leprosy bacillus is made by using the mass of leprosy bacilli isolated from infected armadillos and nude mice. If the cause of difficulty is cultivation of leprosy bacillus, the cultivation of leprosy bacillus would be able to succeed by transformation of DNA.

1. Microbiology of leprosy bacillus

It was clear that insertion of DNA pYA1065 was detected on the 19 positions in DNA of leprosy bacillus. From 8–10 copies of insertion DNA 1.4 Kbase were also detected in the DNA of *Mycobacterium paratuberculosis* that was known as a difficult cultivable acid-fast bacillus. Nobody knows where the DNA fragment comes from, but the DNA fragments are inserted regularly in the host DNA sequence. On the other side, we attempted to produce the antigen of leprosy bacillus by introducing the plasmid of *E. coli* to the leprosy bacillus DNA which was coding the alpha antigen. This trial was not successful.

Mass production of the 65 K protein of leprosy bacillus succeeded by the multicopy plasmid pUC8 of E. coli which was jointed to leprosy bacillus DNA corresponding to the 65 K protein coding.

2. Experimental leprosy

Leprosy bacillus in footpad leproma of BALB/C nude mouse was reduced by transfusion with thymus cell of BALB/C Nu^+ mouse. Minimum infectious numbers of leprosy bacilli were examined by inoculation to BALB/C nude mice, one to five leprosy bacilli can induce leproma in the footpad of the BALB/C nude mouse.

When leprosy bacillus was injected into the different systems of the nude mouse, the leproma size was influenced by genetic factor out of nude gen. Hybrid nude mice of CD-1 x NFS/N or CD-1 x N-NIH(S) produced the biggest leproma. The MRL/1pr mouse permits the growth of leprosy bacilli without nude gen, but IL-2 production may be stopped by the T cell abnormality. Therefore, the MRL/1pr mouse may not be suitable for the vaccine trial test.

3. Chemotherapy of leprosy

Ofloxacin was ineffective to nude mouse leprosy when administered as a food-drug mix, but one spoon-dose a day effectively inhibited growth of leprosy bacillus. Several kinds of ofloxacin derivatives, quinolon derivatives, AM833, AM1091, and AT4140 were effective with nude mouse leprosy.

Since the hybrid nude mouse of Jcl x AF exhibited a fairly effective response to immune-stimulating substances, the effect of immune-stimulating substances MDP (cell wall fraction), CP-46665, ATSO (-1,3 glucan) were tested to suppress the formation of leproma in nude mouse footpad by administration of combination with DDS. DDS alone was not so effective, but a combination of immune-stimulating substances and DDS was effective.

4. Immunology of leprosy

A high-risk group for latent leprosy infection that was selected by high titer of FLA-ABS test and by Mitsuda reaction negative, were treated with antileprosy drugs. This method was applied especially to young family contact.

Leprosy specific antigen (NT-P-BSA, natural trisaccharide-phenyl propionate-bovine serum albumin) was made by joint bovine serum albumin and chemically synthesized trisaccharide. The artificial antigen was used to serum reaction by ELISA method. Serum titer was easily determined by the agglutination of gelatin particle which was jointed to the artificial trisaccharide antigen (MLPA, *Mycobacterium leprae* particle agglutination). Since the serum reaction titer is at a high level in a state of multiplication of leprosy bacilli, the serum reaction was used to determine the effect of chemotherapy, to predict the relapse, and to detect the high-risk group of latent leprosy infection in family contacts.

Mouse peritoneal macrophages were activated with phagocytosis of heat-killed *M. avium* or *M. lepraemurium*, but the expression od Ia antigen on surface of macrophage decreased according to bacterial numbers of phagocytosis.

SIGNIFICANT ACCOMPLISHMENTS

1. Microbiology of leprosy bacilli

Success in the cultivation of murine leprosy bacilli (MLM) was one of the most important achievements. After enzymatic activity was compared between *in vivo* and *in vitro* MLM, it was found that MLM lacked the biosynthetic activity of cyt a and c where cultivated strains of MLM were used. This biochemical characteristic of MLM was not found in other cultivable acid-fast bacilli, and it was presumed to be a cause of difficult cultivation. Since *in vivo* bacilli transform their enzymatic system into that adaptable to their *in vivo* respiration, pursuit of biochemical characteristics only in the *in vivo* bacilli cannot elucidate why they cannot be cultivated *in vitro*. Isolation of pure MLM enabled the analysis of the bacterial components and the purification of the enzymes.

The achievement to be evaluated next was the advance in biotechnological studies. A plasmid (phasmid) which could multiply both in a strain of acid-fast bacilli and of *E. coli* was prepared. The development of a mycobacterial vector could be achieved by binding a plasmid from *E. coli*. This experimental success will become a clue to enable leprosy bacilli cultivation. It is also usable for possible biosynthesis of their protein component inside viable BCG, and, moreover, development of an anti-leprosy vaccine will be aided by this success.

Afterwards, the transduction of a plasmid in *Mycob. gordonae* following the insertion of a kanamycin-resistant gene into a mutant strain of BCG name S10 was examined whose restricted enzyme activity was lower than that of original BCG. As the result, a high frequency in expression of transformed character was observed.

The next achievement to be evaluated was the discovery of a DNA segment, pYA 1065 in the gene bank of leprosy bacilli at 19 sites of DNA of this pathogenic strain using Restriction Fragment Length Polyphornism (RFLP) method. The RFLP pattern was usable for determining leprosy bacilli and identifying them from naturally infected armadillos and mangabey monkeys to be homologous to human source pathogen.

On the other hand, 1.4 K base DNA was found at 8–10 sites in DNA of *Mycob. paratuberculosis*. The presence of this inserted DNA became a common phenomenon found in uncultivable acid-fast bacilli. While the origin of this DNA segment is unknown yet, it is presumed to be doubtless that the insertion occurs under the recognition of DNA configuration to be inserted by this segment.

The lack of catalase activity of leprosy bacilli is said to be proved. Now, anti-catalase antibody is said to bind a catalase protein. It is presumed that a DNA coding high molecular protein such as catalase contains DNA configuration where low molecular protein DNA can insert and similarly, the inactivity of catalase in leprosy bacilli is induced by a dysfunction of DNA due to the low molecular DNA insertion.

2. Experimental leprosy

The successful transmission of leprosy bacilli to armadillos was the greatest achievement in this panel throughout 25 years. Detection of naturally infected armadillos proved that this animal species was the host of leprosy bacilli besides human beings in the nature. Since the collection of a large amount of leprosy bacilli became possible, their biochemical and DNA experiments became facile. It also contributed to an advance in the biotechnology of acid-fast bacilli.

On the other hand, successful transmission of this pathogen to nude mice facilitated the collection of pure leprosy bacilli and the screening of anti-leprosy substances because nude mice were small animals bred aseptically and more favorably than armadillos. The growth inhibitory effect of DDS was found to be incomplete in the nude mouse experiments. Leprosy bacilli from a biopsy material of leprosy patient who had been dosed with 450 mg-RFP only for 2 days did not proliferate in nude mice. It proved their complete eradication. These findings became an origin of multidrug leprosy therapy.

Leprosy was also found in chimpanzees and mangabey monkeys with a considerably high rate of onsets in the latters. Since they are the animals analogous to human being, a pathological analogy of their leprosy to that of human is expected. Nevertheless, the permission to import them is regulated by a Washington, D.C., agreement. The latency of a simian AIDS virus was said to be detected in mangabey monkeys. The advance of studies on a causation between immunological disorders induced by the detected simian AIDS virus and onset of monkey leprosy is expected.

3. Chemotherapy of leprosy

The U.S.-Japan Cooperative Medical Science Program started when DDS was the nearly only reliable drug. However, all the drugs such as DDS, Ciba 1906, streptomycin, and kanamycin could not eradicate leprosy bacilli in lepromatous patients. Moreover, the detection of DDS-resistant leprosy bacilli and relapsed cases brought about a serious difficulty in eradicating the infection source. Therefore, RFP and B 663 were introduced that could eradicate this pathogen in lepromatous cases who had been resisting chemotherapy.

The inhibitory effect of DDS was limited in nude mice, whereas this drug was dramatically effective on this pathogen slowly growing in euthymic normal mice. This result also indicated an only limited efficacy of DDS on lepromatous cases. The similarity of nude mice to lepromatous cases can be understood because the formers are congenitally athymic and so, essentially, lack the cell-mediated immunity.

All of leprosy bacilli in lepromatous patients could be eradicated by the oral dose with 450 mg-RFP for only 2 days. It was ascertained when acid-fast bacilli from lepromas of the patients were inoculated into nude mice. Thus, eradication of the infection source became possible by an immediate dose with RFP when a case was diagnosed to be lepromatous. It also suggested the needlessness of isolating leprosy patients. Leprosy bacilli are detected in the subcutaneous tract of lepromatous cases covering 3–5 years postadministration with RFP regimen. However, they are never viable and so cannot be the infection source of leprosy.

B 663 also acts as a bactericidal drug on this pathogen. Its oral intake promoted the lowering in bacillary index.

A drug examined in the next stage was one of quinolones, ofloxacin, and some further promising quinolones such as AM 1091, AM 833, and AT 4140 are currently being developed.

4. Immunology of leprosy

An antigen specific to leprosy bacilli, phenol glycolipid was found where its terminal 3 saccharides were determined to be specific. Its configuration was Glc-Rha-Rha-phenol-lipid. The synthesis of this determinant was achieved, and its conjugate to bovine serum albumin was developed as an artificial antigen.

In the past, the field survey by a fluorescent leprosy antibody absorption test (FLA-ABS test) indicated the presence of a health high-risk group with a positivity examined by FLA-ABS test and no or nearly no response to Mitsuda reaction due to family contact. This group was monitored to be treated for its preventative purpose.

The development of some ELISA assay methods using this specific antigen is enabling a more specific serum reaction for monitoring this group. Though this antigen is still not enough to become a really reliable one for diagnosis of leprosy, as antibody titers assayed by these ELISA methods augment when leprosy bacilli are proliferating *in vivo*, it is usable for the assessment of efficacy by the dose of chemotherapeutics and the prognosis of possible relapse cases and/or those who have a risk to induce leprosy onset in the future.

Since the biotechnological mass production of IL-2 could become practical, it was applied clinically to patients. The local injection of IL-2 to a leproma of lepromatous patient could form granuloma, resulting in the deformation of leprosy bacilli. Nevertheless, it is localized to the injected site and the disappearance of leprosy bacilli from other lepromas could never succeed. If lepromatous patients can synthesize IL-2 by themselves, the formation of granulomas followed by the deformation of pathogen should be possible. Therefore, the essential cause why a patient is lepromatous type is due to the disturbance of IL-2 biosynthesis. Since IL-2 is produced by T cells, it is reasonable to presume that a T-cell function responding to leprosy bacilli lacks in lepromatous patients.

A membranous protein component of leprosy bacilli has a strong promotive action on the transformation of venular monocytes from tuberculoid patients. Namely, this protein may be a substance inducing cell-mediated immunity.

A possible factor deciding whether a healthy person is induced by a leprous onset or not is still uncertain though it has been studied by various methods.

FUTURE GOALS

Some inserted DNA was found in leprosy and Johne's bacilli, both of which were uncultivable acid-fast bacilli. Moreover, since each DNA was found at a definitive site when examined by RFLP method, it was supposed that the DNA was inserted recognizing the configuration of DNA to be inserted by an unknown mechanism. The insertion occurred at DNA coding a high molecular protein moiety and as the result, the biosynthesis of the protein seemed to become impossible. The insertion was found at 19 sites in leprosy bacilli and 8–10 sites in *Mycob. paratuberculosis*. It suggested the difficulty in cultivating leprosy bacilli is due to these serious deficiencies in their protein synthesis. Herewith, a biotechnological study should emphasize the cultivation of leprosy bacilli by supplementing DNA.

The eruptive endemism of leprosy is decreasing as a result of multidrug therapy. However, the appearance of relapse cases will again be inevitable. Therefore, studies on a possible antileprosy vaccine should be promoted as well as an active endeavor for development of novel drugs. For the former purpose, the first step is to develop a method for isolating a low virulence strain of leprosy bacilli after their cultivation becomes possible. The next step is to prepare an antileprosy vaccine by a strain of viable leprosy bacilli which can induce immunity against this pathogen and are presumed to result from transduction of a protein component controlling the immunogenicity of the low virulent leprosy bacilli into BCG.

On the other hand, it should be expected that the efficacy of an antileprosy vaccine can be examined by the use of euthymic normal mice inoculated with leprosy bacilli.

The mechanism of onset of disease in leprosy is different from that in other infectious diseases. The immunological and epidemiological questions whether a healthy person develops leprosy or not and why his symptom becomes lepromatous should be clarified.

Supply of lepromin antigen: It has become difficult to collect enough lepromas containing leprosy bacilli due to the advance of chemotherapy. Since lepromas of armadillos are apt to be contaminated by various saprophytic microorganisms, the presence of an endotoxin is inevitable. The lepromas of nude mice are free from other acid-fast bacilli and saprophytic microorganisms. A density gradient centrifugation using Ficoll 400 under a high-speed rotation has enabled the collection of pure leprosy bacilli. Thus, the supply of a new lepromin prepared without using chloroform is possible. The development of a new lepromin antigen is indispensable for comparing its antigenic titers with those of present lepromin.

PROJECTS SUPPORTED BY THE PROGRAM

Investigator and Institution	*Title*
Masahide Abe National Institute for Leprosy Research (NILR)	Studies on subclinical leprosy infection—Salivary antibodies in household contacts and inhabitants in endemic area Immunological studies on subclinical infection in leprosy
Tsuyoshi Fujiwara Department of Natural Sciences, Nara University	Chemical synthesis of sugar derivatives of *Mycobacterium leprae* specific phenolic glycolipid and production of sugar protein conjugates The correlation between seroreactivity and molecular structure of sugar moiety of phenolic glycolipids (a preliminary report) Synthesis of PGL-I related trisaccharide of *Mycobacterium leprae* and production of neoglycoprotein conjugates Serological activity of methyl groups in the sugar moiety of PGL

Yasuo Fukutomi NILR	Role of macrophage in protective immunity to Mycobacteria species Macrophage activation after phagocytosis of Mycobacteria species Immunological studies on clinical state of leprosy analysis of lymphocyte subset by flow cytometry
Masaichi Gidoh NILR	Studies on the improvement of immunity in leprosy by drugs (1) Lack of the arthritogenicity of leprosy bacilli derived from nude mouse footpads in rat adjuvant induced arthritis (2) Influence of immunostimulants on the growth of leprosy bacilli inoculated into footpads of hybrid nude mice
Masamichi Goto National Leprosarium Hoshizuka Keiai-en	Immuno-histochemical studies on the *in vivo* distribution and behavior of *Mycobacterium leprae* antigen
Tsunehiko Hirata NILR	Acid-fast organisms isolated and cultivated from the nasal washings of lepromatous leprosy patients: Inoculation experiments to nude mice Re-examination on pyridine extractability of acid-fastness from leprosy bacilli Acid-fast organisms cultivated from the biomaterials in leprosy: Inoculation experiments of the organisms isolated and cultivated from armadillo nodules L154 (456) infected with leprosy bacilli to nude mice Reexamination on pyridine extractability of acid-fastness from leprosy bacilli: Comparative studies of the acid-fastness after pyridine treatment in *Mycob. avium* Kirchberg strain *in vitro* and/or *in vivo* Inoculation experiments of the acid-fast organisms isolated and cultivated from armadillo nodules L154 (456) infected with the leprosy bacilli to nude mice and recultivation experiments of the organisms from the mice Fine structures of the cellular peripheral parts of the leprosy bacilli: Comparative cell morphology with pathogenic fungi and/or non-tuberculous mycobacteria Cytological studies of leprosy bacilli and macrophages in the lepromatous skin nodules of leprosy patients
Tonetaro Ito RIMD, Osaka University	Expression of *Mycobacterium leprae* gene in *Escherichia coli* and *Actinomycetes* Detection of a protein component specific to leprosy bacilli in *Actinomycetes* and the search for its biological activity
Seiichi Izaki Department of Dermatology Iwate Medical University	Studies on the modulative function of granulomatous inflammation using murine leprous mice with special reference to macrophage differentiation and the secretion of proteinase On the differentiation and secretion of macrophages (1) Studies by the use of human cultured cells (2) Studies by the use of human cultured cells and human inflammed granulomatous tissue

	(3) Studies by the use of human and murine cultured cells or by those infected by murine leprosy bacilli (4) Studies by the use of various inflamed chronic granulomatous tissues
Shinzo Izumi Medical Department Kyoto University	Serological property of semisynthetic sugar antigen (NT-P-BSA) and serodiagnosis diagnosis of leprosy Gelatin particle agglutination test for serodiagnosis of leprosy Clinical and seroepidemiological application of *Mycobacterium leprae* specific gelatin particle agglutination test (MLPA) Cell-sorter analysis of the immuneresponse of PBL to *Mycobacterium leprae* antigens Iron metabolism of leprosy bacilli
Yoshiko Kashiwabara NILR	Monoacylphospholipid deacylase activity in leprosy bacilli
Kunio Kawazu NILR	Immunohistopathological analysis of antigens in the leprosy granuloma
Kenji Kohsaka RIMD, Osaka University	Therapeutic effect with thymus transplantation in *Mycobacterium leprae* infected nude mice Therapeutic effect with thymus transplantation in *Mycobacterium leprae* infected nude mice—Sub-inoculation with *Mycobacterium leprae* derived from the thymus transplanted mice Minimal infectious doses of *Mycobacterium leprae* in experimental leprosy of nude mouse Effect of ofloxacin and minocycline on experimental leprosy
Takashi Kusaka Kawasaki Medical School	A rapid and sensitive identification of leprosy bacilli using pyrolysis-gaschromato-mass spectrometry Analysis of ultralong chain fatty acids in leprosy bacilli
Mitsuyoshi Maeda National Leprosarium Tama Zensho-en	Search for an auto-antigen inducing leprous neuritis—Detection of an antigen reactive with a membraneous antigen of Lewis rat Schwannoma cell line Search for an auto-antigen inducing leprous neuritis (II)
Masamichi Makino RIMD, Osaka University	On a d-antigen of *Mycobacterium leprae* with special reference to the biotechnological analysis of antigen determinant specific to *Mycobacterium leprae*
Eiichi Matsuo Medical Department Kyorin University	Studies on the pathological significant of beta-glucuronidase (beta-Gase) in leprosy (1) Especially on its property presumed to be a lipoprotein

	(2) Purification by chloroform treatment, ethanol precipitation, trypsin digestion and gel-filtration, and the property of this enzyme through the behavior found during this purification (3) Conjugation between this beta-Gase and Mycobacterium HI-75. Examination of a possible conjugation between leprosy bacilli and human enzyme suggested by the immuno-histochemical findings of human leprous tissue (4) Conjugation between beta-glucuronidase and a soluble fraction of *mycobacterium* HI-75. Purification of a receptor against beta-glucuronidase (beta-Gase) from *Mycobacterium* HI-75 and the immunological reactivity against this receptor
Yoshiyuki Matsuo Medical Department Hiroshima University	An assay method for examining the viability of leprosy bacilli by a fluorescein staining technique
Masanori Matsuoka NILR	Difference in the bacillary growth of *Mycobacterium leprae* in the footpads of mice among various strains Difference of the host reactions among mouse strains infected with *Mycobacterium leprae* Difference of the delayed-type hypersensitivity reaction among mouse strains infected with *Mycobacterium leprae* Effect of intravenous inoculation with large inoculum of *Mycobacterium leprae* on the bacillary growth in footpad and the host reaction of mice infected with *Mycobacterium leprae* in the footpad Development of the leprosy model with mice
Fumishige Minagawa NILR	Studies on early stage of leprosy infection (1) Development of new type of hemagglutination test, microHA (2) Physiological nature of PGL-I (3) Spreading of leprosy infection in household contacts (4) Simple method for detecting free PGL-I
Yasuhiro Minauchi National Leprosarium Hoshizuka Keiai-en	Examination of humoral factors influential on the disorders by leprous peripheral neuritis (II)
Tatsuo Mori RIMD Osaka University National Leprosarium Tama Zensho-en	Anaerobic cultivation of *Mycobacterium leprae* Collection method of leprosy bacilli from hepatic tissue of infected armadillos; comparison between Draper's and Mori's methods on the transformation of DNA from cultivable mycobacteria to leprosy bacilli Trials for transformation of leprosy bacilli by DNA of cultivable mycobacteria
Kumiko Nagata Department of Bacteriology Hyogo College of Medicine	On the immunological cross over reactivity between *Mycobacterium leprae* and an antigen corresponding to monoclonal antibody against *Mycobacterium tuberculosis* strain Aoyama B or the recombinant peptide antigen derived from DNA of this strain with special reference to the cross over reactivity as infection protective antigens

Atsuo Nakada RIMD, Osaka University	Separation and purification of protein from human leprosy bacilli by gene recombination technique. Studies on the serum and skin reactions using an eptiope of 65 KD protein from leprosy bacilli
Takeo Nakama Medical Department Ryukyu University	Examination of macrophage function and lymphocyte subsets in leprous tissues
Kazunari Nakamura NILR	The effects of genetic background (leprosy-prone) against the formation of lepromatoid lesions in various nude rats
	Leprous lesions at early stage in *Mycobacterium leprae*-infection of nine banded armadillos (*Dasypus nonemcinctus*)
	Leprous lesions in the tongue, stomach, intestinal canal, Peyer's patches, *et al.*, of nine-banded armadillos (*Dasypus nonemcinctus*) with *Mycobacterium leprae*-infection.
	Experimental inoculation with *Mycobacterium leprae* in NOD hybrid nude mice (NOD x CD-1 *nu*)
	Experimental inoculation with *Mycobacterium leprae* in seven banded armadillos
	Mycobacterium leprae-susceptibility of various hybrid nude mice (continued): The result of CD-1 x NFS/N-*nu* and CD-1 x N:NIH(S) hybrid nude mice
	Mycobacterium leprae-susceptibility of various hybrid nude mice (continued): The results of Jcl:AF hybrid nude mice
	The effects of genetic background (leprosy-prone) against the formation of lepromatoid lesions (continued): The results of KSN nude mice
	Experimental inoculation with *Mycobacterium leprae* in various Wistar rats (continued): The results of WKAH/Hkm rats
	Experimental inoculation with *Mycobacterium leprae* in various Wistar rats (continued): The results of WBN/Kob rats
	Experimental inoculation with *Mycobacterium leprae* in various hairless rats (continued): The results of WB/Kob-*Ht* rats
	Experimental inoculation with *Mycobacterium leprae* in SENCAR mice
	Mycobacterium leprae-susceptibility of various hybrid nude mice (continued): The results of SENCAR hybrid nude mice
	Mycobacterium leprae-susceptibility of various hybrid nude mice (continued): The results of CD-1 x CBA/N-*nu* and CBA/N x CD-1-*nu*) hybrid nude mice
	Experimental inoculation with *Mycobacterium leprae* in various hybrid lupus mice (continued): The results of MRL/*lpr* and NOD hybrid mice
	Mycobacterium leprae-susceptibility of NAR rats
	Experimental inoculation with *Mycobacterium leprae* in SHRSP rats

	Experimental inoculation with *Mycobacterium leprae* in ICR (cataract) rats and effect of nude gene on development of cataract Effect of cyclosporin A against growth of leprosy bacillus
Tetsu Nakayama National Institute for Leprosy Research (NILR)	Trials for cultivating *Mycobacterium leprae* in synthetic media
Yasuharu Nishimura Medical Institute of Bioregulation Kysushu University	Genetic control of immunological response to *Mycobacterium leprae* antigen in mice transduced HLA-class II gene
Hiroko Nomaguchi RIMD, Osaka University	Suppression of lymphocyte proliferation by *Mycobacterium leprae* specific antigen, phenolic glycolipid against the stimulation by Con A or PHA Intracellular cultivation of *Mycobacterium leprae* Trials for cultivating *Mycobacterium leprae*
Hiroko Nakagawa NILR	Studies on the synthetic pathways of glycerolipids in murine leprosy bacilli on a phosphatidylinositol synthetase activity Studies on the degradation pathways of phospholipid in murine leprosy bacilli
Nobutaka Ohsawa Medical Department Kitazato University	Analysis of host (mouse) response to the infection of murine leprosy bacilli with special reference to the infection of macrophages On the activity of liposome-encapsulated chemotherapeutics against cultured macrophages
Toshiharu Ozawa NILR	Clinico-immunological studies on leprosy (1) Immunological studies on clinical state of leprosy analysis of lymphocyte subset by flow cytometry (a preliminary report) (2) Restriction of immunological response by HLA-class II monoclonal antibody (a preliminary report) (3) Assay of lymphocyte subsets in lepromatous patients by an indirect monoclonal antibody rosette method
Hajime Saito Department of Microbiology and Immunology, Shimane Medical University	On the antileprous activities of various antimicrobial agents including new quinolones On the antileprous activities of new quinolones, AM 1091 and AM 833
Nanako Saito NILR	Susceptibility difference among various strains of rats against the infection of murine leprosy bacilli and their immunosuppression mechanism
Yoshimoto Sakamoto NILR	Quantitative analysis of the phenolic glycolipid-I in sera from leprosy patients Quantitative analysis of the phenolic glycolipid-I in sera from leprosy patients (continued) Improvement of the determination for leprosy specific antigens

Takehiko Sasazuki Medical Institute of Bioregulation Kyushu University	Molecular level analysis of HLA-linked immunological unresponsiveness specific to *Mycobacterium leprae* antigen Genetic control of immunological response to *Mycobacterium leprae* antigen in HLA-DQW6 gene-transduced mice
Yoshinori Tanaka Medical Department Tottori University	On the growth of *Mycobacterium leprae* and the immunological function in cyclosporin A-treated mice
Haruaki Tomioka Department of Microbiology and Immunology Shimane Medical University	Studies on behavior of suppressor macrophages induced to mice after vaccinated On the behavior of suppressor macrophages induced by a vaccination and their control by biological response modifier (BRM)
Sadae Tsutsumi NILR	Fundamental studies on the improvement of antileprosy pharmacotherapy (1) Synthesis of thiosemicarbazones (2) Synthesis of adamantane imidazoles (3) Search for inhibitory substances against the growth of leprosy bacilli inoculated to nude mice (4) On the strong growth inhibitory effect of a novel quinolone, AT-4140 Analytical studies on anti-leprosy drugs
Ryuichi Tsuyuguchi Department of Bacteriology Hyogo College of Medicine	On the cross over reaction between leprosy bacilli and monoclonal antibody against *Mycobacterium tuberculosis* strain Aoyama B

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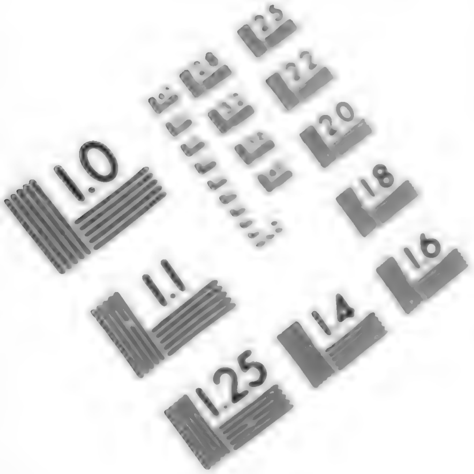

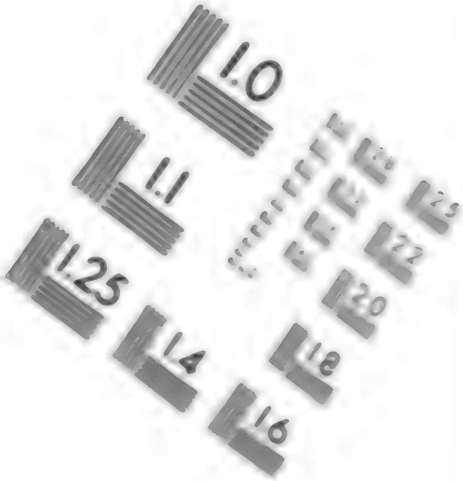

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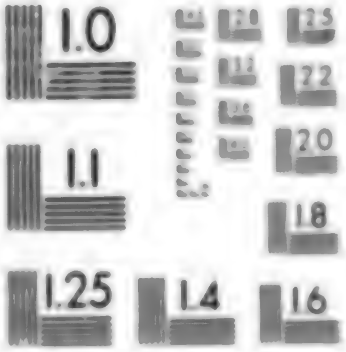

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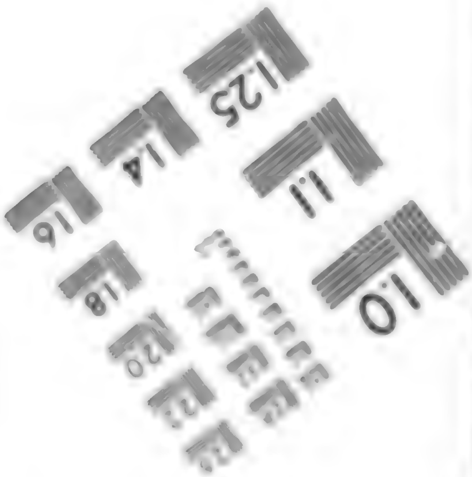

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Joint Scientific Conferences

LEPROSY PANELS

Date: August 12–14, 1985

Place: NIH, Bethesda, Maryland

Summary: The 20th Conference was held in Bethesda, Maryland, at the National Institutes of Health. On August 12 and 13, papers by members and guests of the Leprosy Panels were presented, and on August 14, a joint symposium on leprosy and tuberculosis was held. The subject of the joint symposium was "Recent Advances in Immunology and the Immunology of Leprosy and Tuberculosis." Nine representative papers were presented by the Panel members and guests. The Conference on Leprosy was organized into four separate sessions covering subjects such as: suppressor cell activity in lymphocytes and T cells; isolation of cell wall components; serodiagnosis of leprosy; and animal models for leprosy. A total of 24 papers were presented.

Date: September 25 and 27, 1986

Place: International Hotel, Osaka, Japan

Summary: The 21st Conference was held in Osaka, Japan, at the International Hotel. On September 25 and 27, papers by members and guests of the Leprosy Panels were presented, and on September 26, a joint symposium on leprosy and tuberculosis was held. The subject of the joint symposium was "Molecular Biology of Mycobacteria." Seven representative papers were presented by the Panel members and guests. The Conference on Leprosy was organized into four separate sessions covering subjects such as: microbiology and antileprosy drugs; experimental leprosy; antigens and antibodies; and cell-mediated immunity. A total of 24 papers were presented.

Date: July 20 and 22, 1987

Place: NIH, Bethesda, Maryland

Summary: The 22nd Conference was held in Bethesda, Maryland at the National Institutes of Health. On July 20 and 22, papers by members and guests of the Leprosy Panels were presented, and on July 21, a joint symposium on leprosy and tuberculosis was held. The subject of the joint symposium was "Recent Advances in Immunology and the Immunology of Leprosy and Tuberculosis." Eight representative papers were presented by the Panel members and guests. The Conference on Leprosy was organized into four separate sessions covering subjects such as: serodiagnosis of leprosy; mycobacterial antigens; macrophage function; and epidemiological studies of leprosy. A total of 21 papers were presented.

Date: July 27 and 29, 1988

Place: Nara University, Nara, Japan

Summary: The 23rd Conference was held in Nara, Japan, at the Nara University. On July 27 and 29, papers by members and guests of the Leprosy Panels were presented, and on July 28, a joint symposium on leprosy and tuberculosis was held. The subject of the joint symposium was "Stress Proteins and Antibody Responses to T-dependent Antigens." Seven representative papers were presented by the Panel members and guests. The Conference on Leprosy was organized into four separate sessions covering subjects such as: new antileprous compounds; species specific DNA probes; antigens and antibodies in leprosy; and macrophage activation. A total of 17 papers were presented.

Date: August 23 and 25, 1989

Place: Town & Country Hotel, San Diego, California

Summary: The 24th Conference was held in San Diego, California at the Town & Country Hotel. On August 23 and 25, papers by members and guests of the Leprosy Panels were presented, and on August 24, a joint symposium on leprosy and tuberculosis was held. The subject of the joint symposium was "Specific Detection of Mycobacteria and Vaccine Development." Nine representative papers were presented by the Panel members and guests. The Conference on Leprosy was organized into four separate sessions covering subjects such as: T cell suppression; native proteins; diagnosis of leprosy; and antigen expression in macrophages. A total of 18 papers were presented.

MALNUTRITION PANELS

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Dr. Ryokuero Suzue, Chairman (1984– , Member)
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Profiles

U.S. MALNUTRITION PANEL

Dr. James Allen Olson
Chairman (1989–Present)

Dr. Olson, Distinguished Professor of Sciences and Humanities in the Department of Biochemistry and Biophysics, Iowa State University, has long-term associations with Japan. He studied Japanese in the mid-1940's and worked in Japan for 2 years following World War II. He returned as a visiting professor to Kyoto National University in the mid-1960's, and he has attended many conferences in Japan since that time. He is familiar with Southeast Asia as well, having served as professor and chairman of the Department of Biochemistry at Mahidol University in Bangkok, Thailand from 1966 to 1974. His current research interests concern vitamin A and the carotenoids, relative both to their metabolism, function, and therapeutic actions as well as to their associations with chronic disease.

Dr. Irwin Harold Rosenberg

Chairman (1983–1988)

Doctor Rosenberg is currently Director of the USDA Human Nutrition Center on Aging at Tufts University School of Medicine and Professor of Nutrition within the Tufts University School of Nutrition. He received his undergraduate education at the University of Wisconsin, Madison, and his M.D. from Harvard University in 1959. Following internship and residency at the Massachusetts General Hospital in Boston, he became a research associate at the National Institutes of Health where he was involved in clinical research on malnutrition and cholera in East Pakistan as well as conducting other research investigations. In 1964 he returned to Boston as a research fellow and subsequently a faculty member at Harvard Medical School. He moved to the University of Chicago in 1970 and soon he became Chief of the section on gastroenterology. He was the initial director of their clinical nutrition research center, a position he held until he accepted his current position in 1986. Throughout his career, Dr. Rosenberg has been active in national and international programs concerned with nutrition and health and has made many important contributions to this field. His current research interests pertain to intestinal absorption and transport of vitamins and minerals with a special interest in the metabolism and transport of folic acid. Doctor Rosenberg was a member of the U.S. Malnutrition Panel from 1978 through 1988 and Chairman from 1983 to 1988.

Dr. David Baird Coursin

Chairman (1974–1978)

Doctor Coursin currently serves as a consultant on nutritional issues and maintains clinical appointments on the staffs of several hospitals including Georgetown University School of Medicine. He received his undergraduate education at Haverford College, Haverford, Pennsylvania, and his M.D. at the University of Pennsylvania. After completing a pediatric internship and residency he became involved in research studies identifying the relationship of vitamin B6 (pyridoxine) deficiency in human infants to brain function, convulsive seizures, and behavioral and mental retardation. Observations stemming from his investigations contributed to the formulation of dietary allowances and international standards for vitamin B6 in the diet. His research lead to his interest in identifying the effects of malnutrition on human brain development, behavior, and performance. This research interest blended well with the initial concerns of the U.S.-Japan Malnutrition Panel on which he served from 1967 through 1974 and was Chairman from 1974 to 1978. His efforts in this area did much to strengthen the U.S.-Japan relationships concerning the impact of malnutrition on development and behavior.

Dr. Nevin Stewart Scrimshaw

Chairman (1965–1974)

Doctor Scrimshaw is currently Institute Professor Emeritus at the Massachusetts Institute of Technology and serves as the Director of Food, Nutrition, and Development Program within the United Nations University. He also maintains active appointments at Harvard University, Tufts University, and Brown University. He received his undergraduate education at Ohio Wesleyan University in 1938, his Ph.D. in Physiology from Harvard University in 1941 and his M.D. from the University of Rochester in 1945. Throughout his career, Dr. Scrimshaw has been extremely active in participating in both national and international organizations concerned with nutrition and public health. He has served as an adviser to most of the Federal agencies that deal with nutritional problems and has received numerous honors for his efforts from these organizations. At the time the U.S.-Japan Malnutrition Panel was formed, he was a consultant to the Office of War on Hunger where he helped to develop measures to meet the emergency and chronic and nutritional needs of infants, preschool children, and pregnant and nursing mothers in India. His active efforts concerning nutrition and development made him an ideal candidate for the first chairman of the Malnutrition Panel within the U.S.-Japan Cooperative Medical Science Program. He remained Chairman of this panel from 1965 to 1974. Following his active participation within the Malnutrition Panel, he was a member of the U.S. Delegation to the U.S.-Japan Cooperative Medical Science Program from 1975 to 1985.

Doctor Scrimshaw was instrumental in setting the initial priorities for the U.S.-Japan Malnutrition Panel which included the interactions of nutrition and infection, prevention of nutritional anemias, effects of malnutrition on learning and behavior, investigations of environmental and host factors affecting nutritional requirements, and improvements in the availability of quality dietary protein. Many of these priority areas continue to serve as a foundation for the present priority areas identified by the panel. Through his efforts, the Malnutrition Panel of the U.S.-Japan Cooperative Medical Science Program made major influences on human and public health nutrition.

JAPANESE MALNUTRITION PANEL

Dr. Ryokuero Suzue

Chairman (1984–)

Dr. Suzue was born in April 1925 in Kyoto, and graduated from Kyoto University, Faculty of Medicine in 1951. After receiving his Ph.D. degree in 1957, he spent 2 years at Indiana University. In 1961, he was promoted to Associate Professor in Kyoto University and then moved to the National Institute of Nutrition. In 1981, he was appointed Director General of the National Institute of Health and Nutrition. His research field is vitaminology, and he has been Chairman of the Malnutrition Panel since 1984. He was also Chairman of the Japanese RDA Committee in 1989.

Dr. Goro Inoue

Chairman (1978–1984)

Dr. Inoue was born in July 1917 and graduated from Kyoto Prefectural University of Medicine in 1941 where he was promoted to Associate Professor in 1951. After getting a Ph.D., he moved to Osaka City University and then Tokushima University as a professor of nutritional physiology. After retirement from Tokushima University, he was appointed professor of nutrition at Nakamura University and later moved to Koshien University. His research fields are protein and amino acids requirements for humans and also protein metabolism.

He has attended several international meetings including WHO and FAO, and he was appointed a member of WHO Expert Advisory Panel on Nutrition. He was also a Chairman of Malnutrition Panel, U.S.-Japan Cooperative Medical Science Program from 1978 to 1984. He is now an Emeritus Professor of Tokushima University.

Dr. Hisato Yoshimura

Chairman (1977–1978)

Dr. Yoshimura was born on February 9, 1907, and graduated from Kyoto University in 1930. He was a research associate in Department of Physiology in Kyoto University where he earned a Ph.D. in 1936 and was promoted to Assistant Professor and then to Associate Professor in 1946. He moved to Kobe University in 1946, and then to Kyoto Prefectural University of Medicine in 1947 as a professor of physiology. In 1935, he went to Harvard University and University of New York to study human physiology and nutrition. In 1967, he was appointed as a resident of Kyoto Prefectural University of Medicine and retired in 1972. He received a prize of nutrition from the Japanese Society of Food and Nutrition for an article entitled, "Studies on Recommended Allowances of Protein." His hobbies are skating and archery. He was a Chairman of Malnutrition Panel from 1977 to 1978. He is now Emeritus Professor of Kyoto Prefectural University of Medicine.

Dr. Norio Shimazono

Chairman (1966–1977)

Dr. Shimazono was born on March 16, 1906, graduated from the University of Tokyo in 1929, and obtained a Ph.D. degree in 1942. In 1947, he was invited to Niigata University as Professor of Biochemistry. In 1952, he returned to Tokyo and was appointed Professor of Biochemistry at the University of Tokyo. In 1966, he resigned from the University of Tokyo and was conferred the title of Professor Emeritus at the University of Tokyo. Then he served as professor of biochemistry in Tokyo Medical College and Wayo Women's University. He received many prizes from several scientific societies. His life's work has been the biosynthesis of ascorbic acid. He served as a chairman of the Japanese RDA Committee in 1969 and also as Chairman of the Nutritional Council in the Ministry of Health and Welfare. He was also Chairman of the Japanese Malnutrition Panel from 1966 to 1977.

GUIDELINES

MALNUTRITION PANELS
United States-Japan Cooperative Medical Science Program

It is recognized that nutrition-related disorders are serious problems in Asia as well as other parts of the world. During a review of the U.S.-Japan Joint Malnutrition Panels, there was a gradual, but perceptible, shift in emphasis to increasing attention to nutritional concerns of the United States and Japan, which will subsequently impact on the nutritional problems of other countries in Asia. Of particular concern is the health consequences of different and changing dietary patterns and food habits. Dietary patterns have been recognized as a contributing variable or risk factor for a number of chronic diseases. With the current influences of the Westernized diet on food habits in Japan, evidence relating dietary components to chronic disease through basic, clinical, and epidemiological investigations would be of great importance for both countries. To accomplish this task, it would be of value to have improved measures of nutritional assessment that may serve as prognostic indicators of the positive and negative health consequences of certain dietary habits. The following areas have been identified for developing a collaborative research program:

I. Health consequences of changing dietary patterns on health, development of disease, and disease prevention.

- A. Fatty acids metabolism, including unsaturated fatty acids, in diet and health
- B. Bone disease in relation to diet
- C. Effect of sulfur amino acids

II. Nutritional assessment.

III. Endogenous mediators of nutritional metabolism, not limited to mediators generated by inflammation and infection, but also mediators associated with other types of diseases, such as malignancies.

U.S. Malnutrition Panel

PROGRESS IN PRIORITY AREAS

The past 5-year period initiated a new phase of cooperation between nutritional scientists in the United States and Japan. In the past, the major Program goal concerned malnutrition in Southeast Asia, including problems of vitamin A and iron deficiency, maternal and child health, and both behavior and work performance as a function of nutritional status. As a result of the detailed 5-year Program review of 1984–85, however, increased emphasis was placed on nutritional issues of direct concern to the United States and Japan; namely, the assessment of nutritional states other than clinical deficiencies and the interrelationships between nutrient intake and various chronic diseases. Because dietary patterns and lifestyles significantly differ between our two countries, comparative studies, both of experimental and epidemiological types, were fostered.

Thus, the five primary cooperative areas currently are: nutritional assessment, polyunsaturated fatty acids (including broader areas of lipid interaction), endogenous mediators, calcium and osteoporosis, and antioxidants in relation to oxidative stress. As in the past, teams of 1–2 members from each panel, who possess particular expertise in a given area, work together both to design symposia and to foster intercultural interaction.

Annual symposiums, held alternately in Japan and in the United States, have remained a key activity of the Joint Panels. These symposiums have related directly to a major cooperative area, and, in addition to panel members, have involved leading American and Japanese scientists in the Program. Furthermore, many younger Japanese and American scientists have become involved in the symposiums by giving platform or poster presentations and by attending informal symposium events. As a general rule, the symposiums have been held in association with a relevant international meeting; for example, our most recent symposium on cytokines and metabolism was held in New York in conjunction with an International Conference on Micronutrients and Immune Functions. These fruitful associations not only allow our Panel members to interact with a broader group of topics and scientists but also inform many leading international scientists about Panel activities. Most symposium papers have been printed, either as a monograph or as a supplement to a leading nutritional journal.

The symposiums have served as a catalyst for many other cooperative activities, including the participation of members of both Panels in yet other international meetings, exchange visits, and cooperative research activities. The active collaborative efforts between Professor William Lands and Professor Yuichiro Goto and between Professor John Anderson and Professor Takuo Fujita deserve special mention in this regard.

The shift in emphasis of topic areas has also led to changes in the membership of the U.S. Panel. Professor Irwin H. Rosenberg, a distinguished Panel member for a decade and its dynamic chairman since 1983, resigned in early 1989 because of the press of new commitments. Professors Barbara A. Underwood and James D. Cook, after active, exemplary service for over a decade, were replaced by Professors Gerald Keusch and Lawrence Raisz, experts in the fields of endogenous mediators and of osteoporosis, respectively. At the National Institutes of Health, program activities remain in the capable hands of Dr. Van S. Hubbard and Dr. Nancy B. Cummings, under the overview of Dr. Jay Hoofnagle, Director of the Division of Digestive Diseases and Nutrition, NIDDK.

SIGNIFICANT ACCOMPLISHMENTS

In 1966, 1 year after the initiation of the U.S.-Japan Cooperative Medical Science Program, the U.S.-Japan Malnutrition Panel was established with Prof. Nevin Scrimshaw as Chairman of the U.S. panel. The primary purpose of the Panel was to conduct research to help alleviate malnutrition in Southeast Asia. The first set of priority areas were: 1) the effect of malnutrition on mental development of the infant, 2) synergistic interactions between malnutrition and infection, 3) nutritional causes of anemia, and 4) genetic improvement of plant protein sources.

The major focus of Panel activities clearly was *undernutrition*, and more specifically, the physiological effects of inadequacies of protein, energy, vitamin A, iron, other minerals, iodide, and several of the water-soluble vitamins. Because young children are most affected by nutritional deficiencies, maternal and child health also became a primary concern.

Through the efforts of Dr. Donald Whedon, NIH funds were set aside to foster international research activities in these priority areas. An early major undertaking was the establishment, jointly with the Thai Government and the Rockefeller Foundation, of a Malnutrition and Anemia Research Center in Chiangmai, Thailand, under the direction of Prof. Robert E. Olson, then of St. Louis University. In that center, numerous studies were conducted over an 8-year period on protein-energy malnutrition, the etiology of anemia, nutrition and immunity, vitamin A deficiency, and other priority areas. During the same period, many other funded studies were also conducted in Indonesia, in the Philippines, and in other Southeast Asian and Third World countries.

In 1976, however targeted funding for the Program, the so-called R22 grants, was discontinued. Thereafter, fewer research studies on malnutrition were funded by the NIH in Southeast Asia, primarily because of the increased domestic competition for research funds and of the constraints inherent in developing a Third World proposal that would be favorably reviewed through normal study section mechanisms. Greater emphasis was consequently placed by the Panel on annual symposiums (or workshops) as a means of fostering the Program goals.

Priority areas also shifted. In 1980, for example, they were: 1) influence of environmental and host factors on nutritional requirements; 2) health significance and methods of preventing

Young child with protein calorie malnutrition in Northern Thailand.

Iodine-deficiency in Northern Thailand.

iron deficiency; 3) interaction of nutrition, immune competence, and infection; 4) effects of nutrition on physical and mental development, behavior, physical capability, and work performance; and 5) health consequences of different (and changing) dietary patterns and food habits. During the period 1980–84, successful workshops were held on the effects of nutrition on human growth and development (1980), on nutritional requirements in Asian countries (1981), on subclinical iron deficiency (1982), on vitamin A and cancer prevention (1984), and on calcium metabolism and aging (1984).

With the improved nutritional status of populations in much of Asia from 1966–84 and with the growing awareness that the United States and Japan clearly had different interests in Southeast Asia, an historic change in Panel priorities occurred in 1985. Abetted by a detailed 5-year review of Panel activities conducted in 1984–85, the major focus shifted from problems of *undernutrition* in Southeast Asia to comparative studies of nutritional problems common to the United States and Japan. As a corollary of this change, increased attention was given to the relationship of nutrition to chronic diseases that are common to both countries and to basic mechanisms underlying nutritional actions. Thus, "malnutrition" was broadened to include overnutrition, nutritional imbalances, and their possible long-term consequences on health and survival.

The five current priority areas are: nutritional assessment, polyunsaturated fatty acids (including broader areas of lipid interaction), endogenous mediators, calcium and osteoporosis, and antioxidants in relation to oxidative stress. As in the past, experts in each of these area are members of both Panels. Annual symposiums, usually associated with international conferences, deal with state-of-the-art papers on the selected topic. Young scientists from both countries are involved in these meetings, and the symposiums are published to reach a yet broader audience. Professional interchange, cooperative research projects, and other joint activities are fostered. Interactions among Panel members from the United States and Japan have also influenced professional and scientific activities in a much broader sphere, such as in the International Union of Nutritional Sciences and in international and regional congresses that have been held both in Asia and in North America. In essence, current Panel activities are fully in keeping with the research activities and interests of both Japan and the United States.

MARKED ACHIEVEMENTS

Because program priorities changed from an emphasis on undernutrition in Southeast Asia (1966–84) to a concern for nutritional problems common to the United States and Japan (1985–present), the achievements are of two different kinds.

During the first phase (1966–84), a major project of the U.S. Panel was the Malnutrition and Anemia Research Center in Chiangmai, Thailand. This center brought together a significant number of able young scientists both from the United States and Thailand. An excellent, well-equipped institute was built to house them. Concomitantly, under the auspices of the Rockefeller Foundation, a Ph.D.-level graduate research program in the life sciences was initiated at Mahidol University, and a community health program was fostered at the adjoining, new Ramathibodi Hospital in Bangkok. The worthwhile clinical investigations conducted in Chiangmai and the innovative basic research activities developing in Bangkok harmonized to create a new generation of research and clinical scientists in Thailand.

The ultimate impact of those two successful activities on improvements in nutrition and public health in Thailand has been enormous. As one indication of its overall effect, Prof. Aree Valyasevi, a one-time R22 grantee, former dean of Ramathibodi Hospital and the founder and former director of the National Institute of Nutrition, was selected to give the coveted McCollum International Lecture on "Nutrition Research and its Application in Developing Countries" at the International Congress of Nutrition in Seoul, Korea, in August 1989. It is highly unlikely that Prof. Aree would have been so successful in his efforts in the absence of the activities of the U.S.-Japan Malnutrition Panel and of the Rockefeller Foundation.

On a more purely scientific tack, new indicators were developed under the aegis of the Panel for assessing marginal states of several nutrients, including iron and vitamin A, and for determining the extent of the public health problem for several of these nutrients in Southeast Asia. Together with the International Vitamin A Consultative Group and the International Nutritional Anemia Consultative Group, in which U.S. panel members have played a key role, the U.S.-Japan Panel has been instrumental in reducing the prevalence of these nutritional diseases in Southeast Asia.

Even negative findings have utility in a public health context. The observation that rice fortified with several amino acids did *not* improve the growth of children in northern Thailand convinced public health officials in Thailand *not* to allocate precious, limited funds to a large, expensive fortification project. Later, the results from Thailand were confirmed by similar projects conducted elsewhere.

Although more difficult to quantitate, the importance of personal interactions among Asian and American scientists must be cited. Regardless of the results or impact of a given study, friendly professional interactions in the long run lead to improved research activity, teaching, and career opportunities. This situation is particularly true for often isolated scientists in Southeast Asia. The current leaders of nutrition in Asia, and even in Japan, were stimulated, often at a critical time in their career, by nutritional scientists from the United States and, of course, from other industrialized countries. The U.S.-Japan Malnutrition Panel played an important role in fostering such interactions.

During the second phase (1985–present), major achievements are more in the making than completed. An ongoing study that should yield information of significant interest is the relationship between calcium intake and osteoporosis in the United States and Japan. Although both calcium intake and osteoporosis are lower in Japan than in the United States, the reasons underlying these marked differences are not clear. Furthermore, Japanese ingest much more polyunsaturated omega-3 fatty acids and less saturated fat than Americans. Japanese also suffer more from cerebral bleeding and less from heart attacks than Americans, but the percentage of deaths due to cardiovascular disease is about the same in each country. Whether these associations are indirect manifestations of different lifestyles or cause-effect relationships is currently being probed. Thus, the fostering of well-controlled comparative studies in the two countries should be a major achievement of the Program.

Cooperative efforts on basic mechanisms of oxidative stress and endogenous mediators as well as on the development of new indicators of nutritional status should also be most fruitful.

CURRENT AND FUTURE GOALS

The five current priority areas are all important, state-of-the-art topics in nutritional science. They are also central to an improved understanding of the relationship between nutrition and chronic disease. Furthermore, all have attracted the interest of leading scientists and active research groups in both countries. Thus, the five current priority areas will probably continue without significant modification for several years.

Annual symposiums will continue to serve as a major means of stimulating professional interaction and of summarizing state-of-the-art information and concepts about priority areas. A symposium on regulatory mechanisms in bone metabolism will be held in Kobe, Japan, in late October 1990, and another on biotechnology in nutrition is being planned for 1991 in Bethesda, Maryland. As in the past, these papers will be published.

Collaborative research efforts and interactions between American and Japanese scientists are being strongly fostered. Two such joint projects have been initiated within the Panel, and others are in the offing. American and Japanese scientists now participate actively in international meetings held in both countries. Young American and Japanese scientists are also being encouraged to develop such intercultural professional ties, relationships that might well continue for a lifetime.

Funding for cooperative international research has been in short supply for the past decade. Happily, the situation is improving, in part due to the activities of the National Science Foundation in the United States, the Japan Society for the Promotion of Science, and private foundations. The NIH, besides its intramural international fellows program, also fosters some disease-related international activities. Current priority areas should attract the interest and support of these groups.

The outlook for the next 5 years, therefore, is promising. Through its activities, the U.S.-Japan Malnutrition Panel should continue to play a significant role in fostering scientific cooperation and professional interaction among nutritional scientists in the United States and Japan in the future.

PROJECTS SUPPORTED BY THE PROGRAM

GRANTS

Investigator and Institution	*Title*
G.J. Anderson Oregon Health Sciences University	Dietary n-3 fatty acids: transport and uptake into brain
J.K. Beckman Vanderbilt University	Effects of dietary fat on lipid peroxidative injury
N.J. Benevenga University of Wisconsin Madison	Effect of age on sulfur amino acid metabolism
B.R. Bistrian New England Deaconess Hospital	Nutritional determinants of lem production
R.S. Chapkin Texas Agri and Mech University College Station	Dietary significance of gammalinolenic acid
G.F. Combs, Jr. Cornell University Ithaca	Antagonism of selenium function by mercaptans
W.E. Connor Oregon Health Sciences University	Essentiality of dietary omega-3 fatty acid in primates
P.R. Dallman University of California San Francisco	Developmental aspects of iron nutrition
H.F. DeLuca University of Wisconsin Madison	Metabolism and function of the fat-soluble vitamins
R.A. Di Silvestro Ohio State University	Marginal copper intake effects-SOD & lipid peroxidation
J.A. Gelfand Tufts University School of Medicine	Pathobiology of intravenous lipid emulsions in children

D.H. Hwang Louisiana State University Medical Center	Effects of dietary linoleate on arachidonate
S.B. Heymsfield St. Luke's-Roosevelt Hospital Center	Ethnicity and obesity—new composition models
J.D. Kopple Los Angeles County Harbor-UCLA Medical Center	Taurine nutrition in patients undergoing TPN
W.E. Lands University of Illinois at Chicago	Dietary fat effects on eicosanoid production
A. Leaf Massachusetts General Hospital	Mechanisms of disease prevention of n-3 fatty acids
J.A. Olson Iowa State University of Science and Technology	Bioactivity of water-soluble forms of vitamin A
L.G. Raisz University of Connecticut Health Center	Factors influencing bone metabolism
L.G. Raisz University of Connecticut Health Center	Pathogenesis and prevention of osteoporosis
H.W. Sprecher Ohio State University	Dietary fat: physiological function
H.W. Sprecher Ohio State University	Metabolism of (n-3) fatty acids
G.H. Spurr Medical College of Wisconsin	Energy-nutrition of urban women in a developing country
A.L. Tappel University of California Davis	Nutritional function and biochemistry of selenium
A.L. Tappel University of California Davis	Free radical lipid peroxidation damage
P.D. Whanger Oregon State University	Effects of Se intake on human blood and urine fractions
V.R. Young Massachusetts Institute of Technology	Dietary protein intake and human protein metabolism

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Japanese Malnutrition Panel

BROAD GOALS

The overall goals of the Malnutrition Panel are to eradicate malnutrition in the world and prevent chronic diseases in adults. For these purposes, both the U.S. and Japanese Panels agreed to adopt the following five priority areas: 1) nutritional aspect of bone diseases; 2) nutritional assessment; 3) polyunsaturated fatty acids; 4) endogenous mediators; and 5) sulfur containing amino acids.

PROGRESS IN PRIORITY AREAS

Nutritional Aspects of Bone Diseases

The aim of this area is to examine any possible relationships between dietary calcium and the bone status of old women. To examine any possible relationships among dietary and life-style factors and the bone status of Japanese women in two different life periods, a cross-sectional study was undertaken. Age showed a strong, negative correlation with all bone parameters and for the postmenopausal group, lactation length was negatively associated with bone parameters. Analysis of 3-day food diaries showed a low overall mean intake of dietary calcium in both groups, 467 mg Ca/day compared to the recommended level of 600 mg/day. There were positive correlations between higher past calcium intake and bone parameters.

As risk factors for osteoporosis, female sex, menopause, fair skin, insufficient muscle mass, low body weight, low peak bone mass, early menopause, insufficient exercise, low calcium intake, smoking, drinking water, drinking alcoholic beverages, high protein, high phosphorus, high Na, fiber-rich diet, and low sunshine exposure have been suggested. In a nutritional survey on Japanese women aged 35–40 and 55–60, women living in agricultural villages were found to take less calcium both in the past (12–15 years of age) and at present than those living in the city. Although bone mineral content at the radial diaphysis was similar between the two districts in the younger age group, a significantly low bone mineral content was found in women aged 55–60 living in agricultural villages compared to their counterparts in the city. Bone mass tended to be higher in women actively engaged in exercise than in those leading a sedentary life. Calcium intake and exercise appear to be important risk factors for osteoporosis.

A comparison between Japanese and U.S. populations was carried out. In 1986, University of North Carolina-Kobe University cooperative survey was conducted on the nutritional intake and bone mass of the pre- and postmenopausal women age 35–40 and 55–60 around Kobe. Both groups of women, especially the postmenopausal group, showed lower radical bone mineral density than caucasians of corresponding age in North Carolina.

Vitamin D-deficient rats were constantly infused with 50mg 100H/day of 1,25(CH)$_2$D*3* using osmotic minipump. These results demonstrated that 24, 25(OH) 2D*3*, without conversion into 1,24,25(OH)3D*3* accelerates the metabolic clearance of 1,25(OH)2D*3*, and suggested that 24, 25(OH)2D*3* can have therapeutic value for the treatment of hypercalcemia and hypercalciuria induced by 1,25(OH)2D*3*. Effect of ascorbic acid (AsA) deficiency on the regulation of 25-hydroxyvitamin D*3*-1a-hydroxylase (1 alpha OHase) was examined using genetically AsA-deficient osteogenic disorder (OD) rats. Renal 1 alpha OHase activity and serum 1,25(OH)2D level in response to parathyroid hormone (PTH) infusion or PTH plus calcitonin (CT) infusion were markedly suppressed in thyroparathyroidectomized OD rats. Serum calcium was not different in any groups between OD and control rats. These results demonstrated that renal 1 alpha OHase activity is suppressed in AsA-deficient OD rats both without stimulation and after stimulation with PTH and CT, and that the effect of AsA deficiency is not mediated by changes in serum Ca or Pi levels. It is suggested that AsA plays an important role in the regulation of renal 1 alpha OHase activity similar to its role in the maintenance of adrenal mitochondrial steroid hydroxylase.

Studies of calcium and aging was carried out using aortic calcification. In a clinical study, significant relationship between aortic wall stiffness and radical bone mineral content was identified. The stiffness of the aortic wall is known to increase with aging and the phenomenon is considered to result mainly from the accumulation of calcium in the wall. On the other hand, bone mineral content is known to decrease with aging. In this study, a significant negative correlation was found between the aortic wall stiffness and calcium content in the bone. This suggests that the decrease in bone mineral content could be a risk factor for the increase in the aortic wall stiffness.

Reevaluation of recommended dietary allowance of calcium intake in elderly Japanese women was studied. Calcium intake is an important factor in the development of osteoporosis. The recommended dietary allowance (RDA) of calcium intake in Japan is considered to be 600 mg/day. In order to reevaluate RDA in the elderly Japanese women, the calcium balance study was performed in 13 subjects; 6 with osteoporosis (age 75 ± 2) and 7 without osteoporosis (age 72 ± 2). Calcium balance in the individual subject was evaluated by measuring the intake of calcium (food) and the output (feces, urine, and skin loss) every 2 or 3 days. The mean requirement for all subjects was 595 ± 144 mg/day (mean ± SD). The value for the subjects with osteoporosis was higher than for the subject without osteoporosis (640 ± 138 vs. 543 ± 166 mg/day). RDA was calculated to be 832 mg/day. These results suggested that more calcium intake is necessary to maintain the positive calcium balance in the elderly Japanese women, especially those with osteoporosis.

Nutritional Assessment

Assessment of nutritional status including clinical studies for total parental nutrition were studied. The A-mode type ultrasonic device for measuring fat thickness can measure subcutaneous fat thickness more accurately than the skinfold caliper method. The estimation formula using data of ultrasonics was improved as follows. Body Fat Mass $= a_1X_1 + a_2X_2 + b$

X_1X_2: subcutaneous fat thickness (mm) at body sites
a_1, a_2, b: parameters estimated by sex and age

The parameters, a_1, a_2, b_1 are estimated by multiple regression analysis of practical data which measured both of subcutaneous fat thickness and girth length in each of 34 male cases and 34 female cases.

A survey on obesity in occupations—residential and professional factors were studied. A large number of data for subcutaneous fat thickness measured by ultrasonic device were collected in order to establish a new standard of obesity which would be more reliable than skinfold caliper measurement. Subcutaneous fat thickness and clinical examination was done in two districts, i.e., Hase, Kami-Ina, Nagano Prefecture on an agricultural and rural population and Ota-ku, Tokyo on urban merchants who were members of the Merchant Union in 1988.

Results are as follows:

1. In rural populations which are agricultural, workers have less subcutaneous fat than urban merchant populations.
2. The differences could be caused by accumulative effects of residential and professional factors, so that it is impossible to identify a clear effect of any single factor.
3. In spite of the results mentioned above there is little difference among these populations as to Body Mass Index (BMI) which is calculated using body height and weight without any measurement of subcutaneous fat mass.
4. The relationship between subcutaneous fat thickness and clinical examination data is not always clear as related to sex or habitation.

A survey of nutritional status for aged people was carried out in August 1985 at Kyoto. The subjects were 30 males and 19 females. The intake of the nutrients was almost normal for the dietary allowances of this age. The blood status was normal. A correlationship between blood pressure and salt intake could not be found. The energy intake was almost matched by energy expenditure as measured by the pedometer. The nitrogen balance was slightly positive in both sexes. It seems that subjects of this survey were healthy and had a good blood picture because of good food habits.

Food intake and physical development in children under welfare care were investigated. The height and weight of 65 boys and 56 girls aged 6–14 were measured monthly from June 1984 to June 1985. Food intake of 55 boys and 51 girls was also investigated during 3 days in August. From the viewpoint of the intake of the food or their body status, the subjects were seen to have a poor nutritional status but from the normal growth rate and positive nitrogen balance, their nutritional status was not abnormal.

Effect of dietary composition on energy metabolism was investigated with special respect to thyroid hormones. Energy metabolism, one of the most fundamental functions of the living body, is affected by various factors. Among the factors, diet is undoubtedly important. Therefore, the significance of dietary composition in changes of serum levels of thyroid hormones was explored in rats fed various synthetic diets. Some important results are as follows: 1. High-carbohydrate, low-protein diet was confirmed to raise serum levels of T_3 and high-protein, low-carbohydrate diet to lower serum T_3 levels. High-fat, low-protein diet also caused a rise of serum T_3 levels. 2. Dietary carbohydrate and fat at a high content changed serum T_3 levels in the same direction, but the mechanisms responsible to cause high T_3 levels might be different between the two macronutrients. The effect of high-fat diets in raising the serum levels of T_3, was detected in 7- and 13-week rats but not in 20- and 40-week animals. But, it can not be concluded whether age or obesity is the more important cause for the response of aged animals to high-fat diet.

Plasma levels of retinol, RBP, TTR, and free holo-RBP were determined in patients with obstructive jaundice (OJ) and in apparently healthy volunteers. The threshold of taste acuity was also determined in patients with OJ during the clinical courses before and after percutaneous transhepatic biliary drainage (PTBD). Plasma retinol, RBP, and TTR levels were significantly decreased in patients with OJ, whereas free holo-RBP levels were not always decreased in comparison to the healthy controls. Taste acuity was also decreased in most of patients with OJ although in two patients, whose plasma free holo-RBP levels maintained within normal limits, taste acuity was well preserved. Impaired taste acuity was rapidly improved in all patients who received PTBD, free holo-RBP levels. Therefore, it is suggested that taste acuity is affected by free holo-RBP from which retinol is delivered through a specific receptor for RBP on the taste buds.

The hepatic uptake of retinyl palmitate-labeled chylomicron and intercellular as well as intercellular transport of vitamin A in rat liver was investigated. It was suggested that, once received by the parenchymal cell, vitamin A was rapidly transferred to the stellate cell and was deposited in the lipid droplets.

Availability of dietary iron in the elderly 60 years of age and older was estimated. Sixty-eight elderly Japanese, whose serum ferritin levels were over 15mg/ml, were selected. Iron stores of almost all the members of the population were considered to be in the range of 300–2,400mg from the serum ferritin levels. Based on the value of basal losses of 14 alpha/kg of body weight/day, the average of iron requirement of the population was calculated to be about 0.8mg/day for males and 0.7mg/day for females. The amount of available dietary iron was estimated by the analysis of daily dietary records. When the iron store was assumed to be about 500mg, the estimated percent of absorbable iron was about 8% and its amount was 0.8–1.0mg/day. When the iron store was assumed to be about 1,000mg, the estimated percent of absorbable iron was about 4.5% and its amount was 0.5–0.6mg/day.

Serum ascorbic acid concentration in a healthy elderly population as related to its dietary intake was studied. Ascorbic acid status was studied in 175 free-living and healthy elderly people aged 63–81, who were not using ascorbic acid supplement. Mean dietary intake for females was 3.2 ± 1.3 mg/kg of body weight/day (n = 96) and was significantly higher than for males, 2.7 ± 1.1 mg/kg/day (n = 79). The mean serum ascorbic acid concentration for females was 1.11 ± 2.3 mg/100ml and was significantly higher than for males, 0.91 ± 0.30mg/100ml. It was estimated that intakes needed to maintain a serum ascorbic acid concentration of 1.0 mg/100 ml would be about 2 mg/kg of body weight/day for females and 3.5 mg/kg/day for males. Smokers had lower serum ascorbic acid levels and lower dietary ascorbic acid intake than did nonsmokers. Of diet, sex, and smoking, ascorbic acid intake was the most important predictor of its serum concentration, accounting for about 16% of its total variation.

Mortality from cardiovascular disease in Okinawa is only about 59% of that of all Japan. Elderly people living on the isolated islands in Okinawa have been studied nutritionally. The effects of eicosapentaenoic acid have been reported, but in some farming areas of Okinawa, such as Izena, fish intake is not high. In Okinawa, the intake of pork which is rich in monounsaturated fatty acids (MUF), is 151% of that of average Japan. MUF is reported to lower LDL of Americans whose total cholesterol level is high. In Izena, HDL cholesterol level (59.9 ± 14.5 mg/dl total cholesterol = 211 ± 39 mg/dl, n = 565) and MUF of both diet (29% of total FA) and serum (16% of total FA) were all high. In some area pork intake was 75 g/day/capita. Thus, an experimental pork diet (100 g/day/capita) was given to 20 volunteers. During 5 days, on average, serum HDL-cholesterol was elevated from 65 to 70 mg/dl ($P<0.001$), but total cholesterol (190 mg/dl) was hardly changed. High intake of seaweeds and soybean, 124% and 123% of average Japan, respectively was also characteristic of Okinawa. Kombu (a seaweed, 200 g wet weight) combined with the pork diet reduced cholesterol from 184 to 178 mg/dl ($P<0.01$). Cholesterol intake in Okinawa and all of Japan was 350 and 300 mg/day, respectively. Very low urinary Na(127 ± 101 mEq/day) and high serum aldosterone (123 ± 104 pg/ml) may contribute to the low incidence of hypertensives in Okinawa.

Nutritional significance of unsaturated fatty acids.

Polyunsaturated fatty acids, especially n-3 fatty acid that was contained in fish oil and effective for cardiovascular diseases, were investigated.

Another study is the role of apoE in the metabolism of lipoproteins containing apoB 100. Apolipoprotein B100 is a constant component of VLDL, IDL, and LDL. Iodinated VLDL, IDL, and LDL were injected intravenously into normal and LDL receptor-deficient rabbits. Results from normal rabbits indicate that apoE is a major determinant of the metabolic rate of VLDL. In receptor deficient rabbits, defective removal of both B and B, E particles in all three lipoprotein classes, coupled with preserved processes of dilapidation, were observed, and can account for the increases in the concentration of apo B 100

Effect of cholesterol-free diet on plasma cholesterol in heterozygous familiar hypercholesterolemia was studied. Seven patients with a mean plasma cholesterol concentration of 323 ± 67 mg/dl were hospitalized in a metabolic ward and kept on a cholesterol-free diet for as long as 11 days without any medication. The content of dietary fat, carbohydrate, and protein was 18%, 69.2%, and 12.8% of calories, respectively. The concentrations of plasma and lipoprotein lipids and plasma apoproteins were determined every 2 days. At the end of the study, plasma cholesterol was lowered by 14.2% from 323 to 277 mg/dl, and low density lipoprotein (LDL) cholesterol by 17.5% from 229 to 189 mg/dl. High density lipoprotein (HDL) cholesterol concentration decreased slightly, from 41 to 35 mg/dl. These results indicated that restriction of the intake of dietary cholesterol is highly effective in controlling plasma cholesterol levels in heterozygous familiar hypercholesterolemia.

Endogenous mediators and nutritional metabolism

Fever, anorexia, decrease of energy production, and other symptoms were reported as host-defense mechanisms against infection. Interleukin-1 and cachectic, known as the endogenous mediators of above reactions, were investigated nutritionally. A high cholesterol diet induces marked alterations of plasma lipoproteins. One of them is that the plasma contains a specific VLDL beta-migrating lipoprotein referred to as beta-VLDL. The lipoprotein has been regarded as an atherogenic one. In the present study, the effect of high cholesterol diet-induced lipoproteins and macrophages was examined. Rat alveolar macrophages collected by bronchoalveolar lavage from a rat fed a high cholesterol diet for 3 months were significantly larger in size than those from a control rat. When macrophages were cultured for 4 hours, the cells from a cholesterol-fed rat secreted more amounts of lipoprotein lipase (LPL) than those from a control rat. In the second experiment, cultured alveolar macrophages from normal rats were incubated for 2 days with the VLDL obtained from rats fed a high cholesterol diet for 3 weeks. Hypercholesterolemic rat VLDL increased markedly the cellular contents of both cholesterol and triglyceride. The VLDL stimulated the secretion of LPL. It is speculated that macrophages residing in the environment rich in hypercholesterolemic lipoproteins induced by high cholesterol feeding take up the lipoproteins and accumulate lipids intracellularly, and that this process is linked with the LPL secretion. Probably such events are of importance in the foam cell formation of macrophages.

Catabolic effects of interferon gamma, interferon beta, PDGF, and TGF beta on lipid turnover in 3t3-L1 cells were studied. The effects on lipid metabolism of cachectic/TNF, IL-1 interferons, TGF beta, and PDGF, which are known to produce closely interacting activities in many tissues, were examined in 3T3-L1 adipocytes. Among the cytokines examined, IFN gamma showed the strongest suppression of LPL activity. IFN beta and PDGF showed less marked LPL suppression. TGF beta showed a suppressive effect on LPL activity, but to a much smaller degree compared with that of INF beta or PDGF. In contrast to these effects on LPL the effects of IFN beta, TGF beta, PDGF, or IFN gamma on lipolysis were minimal, if any. These results suggest that these cytokines cooperate to produce metabolic changes in the host in response to invasions.

Effects of various cytokines on adipocyte conversion of 3T3-L1 preadipocytes were investigated. The effects of cachectic/TNF, IL-1, IFN gamma PDGF, TGF beta on growth and differentiation of 3T3-L1 cells were examined. This fibroblastic cell line can be induced to differentiate into a mature cell type having the biochemical and morphological characteristics of normal adipocytes. At various stages of growth and differentiation, 3T3-L1 cells were exposed to cachectic/TNF or one of the other cytokines for 24 hours after which cytotoxicity or differentiation was evaluated. During log-phase cell growth, cachectic/TNF had no significant effect on cell viability, and the pre-adipocytic cells were also resistant to the cytotoxic effect of cachectic/TNF at the contact-inhibited confluent stage.

However, when cachectic/TNF was added to the cells during induced differentiation, only 20% of the cells survived. After differentiation into adipocytes, cells regained their resistance to cachectic/TNF-induced cytotoxicity. Cachectic/TNF also markedly affected the differentiation of 3T3-L1 cells into adipocytes. When cells in the confluent phase of growth were exposed to cachectin/TNF for 25 hours their subsequent hormone-induced differentiation to adipocytes was inhibited. Like cachectic/TNF, IL-1, IFN gamma, PDGF, or TGF beta also induced suppression of lipoprotein lipase in 3T3-L1 adipocytes; however, contrast to cachectic/GNF, IL-1 and the 3 cytokines had no or very little effect on the viability or differentiation of preadipocyte 3T3-L1 cells. These results indicate that cachectic/TNF may play a normal role in controlling the development of adipose tissue as well as metabolism in mature adipocytes.

Sulfur containing amino acids

Studies on sulfur amino acids, methionine, cysteine, taurine, and glutathione were investigated nutritionally. It has been demonstrated that taurine plays an important role in the function of excitable tissues such as the central nervous system, retina, heart, and that taurine may be an essential nutrient for infants. However, the biosynthetic ability of taurine in adult human subjects is still uncertain, since the effects of exogenous taurine and the efficiently regulated taurine pool make the conclusion from human experiments complicated.

In order to clarify the possibility of taurine formation from cysteine, the sulfur amino acid metabolism in a patient receiving total parenteral nutrition was examined. The patient had part of his digestive tract removed with only the stomach, duodenum, and a small part of jejunum with a jejunum fistula remaining. He had been fed by total parenteral nutrition after the operation. The daily intake of cysteine and methionine was 92 mg and 1,732 mg respectively. The taurine content in blood and urine gradually decreased to one hundredth of normal level at about 100 days after operation and the infusion of glutathione (600 mg per day), possible precursor of taurine, was started at that time and continued for about 3 months. The taurine contents in blood and urine increased during the 3 months of infusion but decreased gradually after termination of infusion and returned to the original levels.

These findings suggest that the taurine biosynthesis from cysteine may be possible in humans, but the rate of synthesis is limited, and supplementation of taurine may be needed for long-term TPN.

The cysteine dioxygenase activity in rat liver is induced by the injection of cysteine or methionine in the presence of permissive quantities of glucocorticoids and also increased by the decrease of dietary protein bioavailability affecting by its amino acid limitation with concomitant increase of urinary taurine. These findings imply that cysteine dioxygenase and urinary taurine may be available as possible indicators of essential amino acid requirements in the rat.

Protein and amino acid compositions of human milk taken from mothers delivering term and preterm were studied. In Japan, few reports concerning human milk composition exist. In this study, the differences in the composition of mature and premature human milk taken from one breast were examined. 1) Material: 70 samples of mature milk from mothers who gave birth to full-term babies and 66 samples of premature milk from mothers whose babies were born before term. 2) Methods: A total of 136 milk samples were divided into 4 groups: Group A consisted of milk taken from 3–5 days after the baby's birth, group B of milk from the 6th to 10th day, group C of milk from the 11th to 20th day, and group D of milk taken from the 21st day after delivery to two months. Then the compositions of mature and premature milk were compared with regard to protein, fat, energy, amino acids, its NPN content, and dry weight. Chronological changes in mature and premature milk in the four groups were also examined. 3) Results: In comparing mature and premature milk, prominent differences were observed only in the concentration of taurine. There were no significant differences between mature and premature milk, but there was a higher protein concentration in premature milk. No significant tendency among the components studied were observed. From previous report, it was suspected that preterm milk would tend to have relatively high protein and low fat concentrations, but only a high taurine concentration and a slightly higher concentration of protein were observed. It was suggested that the discrepancies between previous reports and the present one may be due to the fact that previous studies examined only milk discharged at the end of nursing, while samples were taken at the beginning, midway, and at the end of nursing in the present study.

Effects of L-methionine (Met) addition to soy protein isolate (SPI) diet on tissue free amino acids, particularly sulfur-containing amino acids, were investigated in pregnant rats. The experimental results indicate that the prenatal brain growth was not specifically affected by excess of dietary Met, at least, to the 21%. The concentration of Met in fetal brain was affected by addition of excess Met to the diet, while that of Met in dam's plasma was not. On the other hand, the concentrations of taurine in dam's plasma and fetal brain were not affected by dietary excess of Met. Thus, the significant relation between concentrations in dam's plasma and fetal brain was not obtained in each of Met and taurine.

SIGNIFICANT ACCOMPLISHMENTS

Sparing Effect of Excess Calories for Protein Requirement

In 1985, FAO/WHO/UNU published the recommended allowances of the requirement of protein for humans. This value was lower than the former RDA. To investigate the quantitative effects of energy and N intakes on N utilization, 67 young men were fed low protein diets varying in whole egg protein and energy for 2 weeks. The N balance was markedly affected, not only by the N intake, but also energy intake. From this experiment, the conclusion drawn, using young men with energy and egg protein intakes near maintenance level, is that the efficiency of N utilization was greatly affected by energy intake. The efficiency rate of energy intake upon N balance was estimated to be higher, about 21.7 times in unit change and by 1.3 times in percentage from maintenance than the efficiency of N intake. The increasing rate of NPU with increasing energy intake and the decreasing rate with increasing N intake were also evaluated quantitatively. In short, the protein sparing effect of dietary energy seems to be more important than the protein intake itself.

High Consumption of Vitamin C on Stress

Consumption of Vitamin C is increased by stress. The production of epinephrine is also increased by stress. The activity of tyrosine hydroxylase, the rate-limiting enzyme of epinephrine biosynthesis, in a homogenate of the adrenal gland decreased in scurvy, and recovered after the administration of ascorbic acid. Immunochemical analysis indicated that there was a constant amount of immunochemically precipitatable enzyme per unit of enzyme activity indicating that the increase of enzyme activity, following the administration of ascorbic acid resulted from an increased amount of the enzyme protein.

New Estimation Method for Dietary Protein

It has been established that the new nutritional evaluation of dietary protein quality, along with the sulfur amino acid metabolism could be used as a possible indicator. The cysteine dioxygenase activity in rat's liver is reduced by an injection of cysteine or methione in the presence of permissive quantities of glucocorticoids and is also increased by decreasing dietary protein bioavailability, affected by its amino acid limitation with concomitant increase of urinary taurine. These findings imply that cysteine dioxygenase and urinary taurine may be available as possible indicators of essential amino acid requirements in rat.

Food Survey of Okinawa Area, Longest Life Span Expectancy in the World

Japanese lifespan expectancy is the longest in the world and Okinawa is the best area in Japan. The mortality from cardiovascular disease in Okinawa is only about 50% of the entire country. The elderly people living on the isolated islands of Okinawa have been studied clinically and biochemically since 1977. Therefore, a food survey was performed in Okinawa. The intake of pork which is rich in monounsaturated fatty acids (MUF), is 151% of the average Japanese diet. MUF is reported to lower LDL in Americans with high cholesterol levels. In some areas, pork intake was 75 g/day/capita. Thus, an experimental pork diet (100 g/day/capita) was given to 20 volunteers. During a 5-day period, average serum HDL-cholesterol was elevated from 65 to 70 mg/dl, but the total cholesterol level (190 mg/dl) was hardly changed. The high intake of seaweed and soybean, 124% and 123% of average Japanese, respectively, was also characteristic of Okinawa. Kombu combined with the pork diet reduced cholesterol level from 185 to 178 mg/dl. Very low urinary Na and high serum aldosterone may contribute to the low incidence of hypertension in Okinawa.

Serum Cholesterol-lowering Activity of Human Monocytic Colony-stimulating Factor

Investigators studied the serum cholesterol-lowering activity in human monocytic colony-stimulating factor in seven boys, who were injected daily, over a period of 7 days, with purified native human monocytic colony-stimulating factors. The average percentage decrease was 20%. The most likely mechanisms might be a decrease in cholesterol synthesis or an increase in cholesterol excretion.

FUTURE GOALS

Bone Diseases and Nutrition

A comparative study with Japanese and U.S. postmenopausal and premenopausal women will be carried out. The variables being measured include: bone density; current calcium intake; estimated past calcium intake; and physical activity.

Nutritional Assessment

Standardization of nutritional indicators must be established, and application of new techniques, gas chromatography; mass spectormetry, new techniques for bone density; ultrasound for body composition; and nuclear magnetic resonance; should be used for clinical nutritional studies.

Polyunsaturated Fatty Acids

Omega-3 fatty acids, or essential fatty acids and nutrition, prevention of cardiovascular disease, and relevant developments in prostaglandins will be investigated.

Endogenous Mediator

The emphasis on cytokines and their regulatory role on immune function with special emphasis on *in vivo* observations will be urgent.

Sulfur Containing Amino Acids

This priority area should be changed to antioxidants and nutrition involving sulfur amino acids; rethinoids; carotenoids; tocopherol; certain trace elements such as selenium; and their dietary constituents.

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Joint Scientific Conferences

MALNUTRITION PANELS

Date: July 15–17, 1985

Place: Bethesda, Maryland

Summary: In response to an overall review of the objectives of the U.S.-Japan Malnutrition Panel, a symposium was planned in conjunction with the 20th anniversary of the U.S.-Japan Cooperative Medical Science Program. Topics presented were indicative of future directions of the joint panel and included (1) nutritional significance of unsaturated fatty acids, (2) sulfur containing amino acids, (3) endogenous mediators of nutritional metabolism, (4) nutritional aspects of bone disease, and (5) nutritional assessment. This symposium was planned to help the U.S.-Japan Malnutrition Panels revise their research guidelines to match the available expertise in both countries. In this way, the development of a truly collaborative research program could be facilitated.

Date: September 28–30, 1986

Place: Osaka, Japan

Summary: The priority area of nutritional assessment was highlighted in the symposium on assessment of marginal nutritional status: Basic and clinical consideration. This very successful 2-1/2 day program also included an evening poster session in which many junior investigators from both Japan and the United States participated. This session demonstrated excellent progress and discussions and research oriented interactions. The formal sessions at the symposium included presentations concerning new technologies applied to nutritional assessment and clinical measures of nutritional assessment for populations as well as for individuals. Throughout the meeting, opportunities for new collaborative research in the various priority areas were emphasized. The proceedings of the symposium were published as a supplement to the *Journal of Nutritional Science and Vitaminology* (33: supplement, S1-S48, 1987).

Date: April 27–29, 1987

Place: San Diego, California

Summary: The U.S.-Japan Malnutrition Panel cosponsored the International Symposium on Clinical Nutrition which included sessions on nutrient control of GI hormones, cancer and nutrition, essential fatty acids, protein energy malnutrition, and calcium interactions with osteoporosis and hypertension. Following the international symposium on clinical nutrition, a special workshop was held entitled "International Aspects of Dietary Guidelines." Within this workshop presentations were made representing the dietary guidelines of the United States, Australia, Europe, Korea, India, and Japan. A summation of this symposium was published in *Nutrition Reviews* (46: 237–240, 1988).

Date: September 25, 1988

Place: Tokyo, Japan

Summary: The symposium entitled "Polyunsaturated Fatty Acid in the Aspect of Nutrition and Clinical Medicine" was held as a satellite to the World Congress of the International Society for Fat Research and the Japan Oil Chemists Society. This symposia emphasized the relative influences of omega-3 and omega-3 fatty acids towards lipid metabolism and the development of various clinical diseases. Because of the presence of many other international attendees at the meeting, much interaction occurred toward developing many new scientific relationships.

Date: June 2, 1989

Place: New York, New York

Summary: This symposium on Cytokines and Metabolism was held in conjunction with a New York Academy of Sciences Conference entitled "Micronutrients and Immune Functions." The malnutrition symposium covered topics involving the crossroads of nutrition and immunity, including cytokines and the metabolic effects they mediate. Topics included presentations on the nature and regulation of metabolic responses to cachectin/TNF, and its potential role in pathogenesis of septic shock; interleukin-1 effects on appetite and the effects of dietary fatty acids; effects of various cytokines on adipocytes; alterations of cytokine metabolism in response to tissue injury; changes in cholesterol metabolism in response to various cytokines; and the potential influence of cytokines in the pathogenesis of senile osteoporosis. The papers within this symposium will be published as part of the Annals of the New York Academy of Sciences.

PARASITIC DISEASES PANEL

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Profiles

U.S. PARASITIC DISEASES PANEL

Dr. Bruce Greene

Chairman (1989–Present)

Dr. Greene received his M.D. from Johns Hopkins University. His interest in parasitology was stimulated upon his introduction to schistosomiasis research during an infectious diseases fellowship at Vanderbilt Medical School. He has proceeded to develop his interests in tropical medicine and parasitology as a faculty member at Case Western Reserve University and, currently, as the Chief of the Geographic Medicine Division at the University of Alabama Medical School. His own research centers on the control of onchocerciasis, and involves both laboratory and clinical investigation. He was appointed to the Parasitic Diseases Panel in 1985 and became Chairman in 1989. During this time, the Panel expanded its efforts to stimulate interaction with investigators working on malaria research, as well as schistosomiasis and filariasis.

Dr. Daniel Colley

Chairman (1985–1988)

Dr. Colley received his doctorate in microbiology from Tulane University and did postgraduate training in immunology at Yale. His long-term interest in parasite immunology was initiated by a period of postdoctoral training in Brazil where he was introduced to schistosomiasis research. He has subsequently developed an international research effort from Vanderbilt University dedicated to the study of parasitology and tropical diseases. His studies have centered on the nature of immunopathogenesis and immunoregulation in chronic parasite infection and have encompassed field research in the West Indies, Egypt and Brazil. Dr. Colley joined the Parasitic Diseases Panel in 1978 and served as Chairman from 1985 to 1988. During his association with the Panel, the scientific emphasis continued to shift from descriptive biology of the schistosomes and filarids to consideration of the host-parasite relationship at the immunologic and molecular level.

Dr. Lawrence Ash

Chairman (1978–1984)

Dr. Ash received his doctorate in parasitology from Tulane University, and has worked in filariasis research in New Caledonia and at the UCLA School of Public Health. His discovery that the Mongolian jird would support the development of *Brugia pahangi* and *B. malayi* in the laboratory has spurred filariasis research since the early 1970's, and remains central to current research efforts in this field. Dr. Ash was appointed as a member of the Parasitic Diseases Panel in 1972, and served as Chairman from 1978 to 1984. During his tenure, recognition of the new possibilities for application of immunology and molecular biology to the study of parasitic infections led to expansion of the Program guidelines to include malaria and other vector-borne diseases. Efforts of the Parasitic Diseases Panels during this period also led to increased interaction between U.S. and Japanese investigators and enhanced scientific exchange between laboratories.

Dr. Franz von Lichtenberg

Chairman (1973–1978)

Having obtained his M.D. from the Universidad Nacional of Mexico, Dr. von Lichtenberg spent several years studying and teaching pathology in Central and South America before beginning his long and fruitful association with Harvard Medical School in 1958. His distinguished research career has encompassed several pivotal observations on the pathogenesis of schistosomiasis, including detailed descriptions of the granulomatous process. His devotion to the field of parasitology and tropical medicine is exemplified by his service not only to the Program as a member of the Parasitic Diseases Panel, but also as a consultant to the National Institute of Allergy and Infectious Diseases and the World Health Organization. Dr. von Lichtenberg was appointed to the Panel in 1971, and acted as Chairman from 1973 until 1978. This encompassed a time of increasing interest in parasitic diseases and application of modern scientific technology to the field.

Dr. Paul Weinstein

Chairman (1969–1973)

Dr. Weinstein received his doctorate in parasitology from the Johns Hopkins School of Hygiene and Public Health and subsequently served in the U.S. Public Health Service, where he worked on malaria control and diagnositic parasitology. He later joined the Laboratory of Parasitic Diseases at the National Institute of Allergy and Infectious Diseases, National Institutes of Health, later becoming chief of that laboratory. He left the NIH to take the chairmanship of the Department of Biology at Notre Dame University. Dr. Weinstein's contributions to the field of parasitology include the development of *in vitro* culture systems for nematodes that facilitated the study of developmental biology in these organisms and allowed elucidation of their metabolic and physiologic pathways. He served as a member of the U.S. Parasitic Diseases Panel from 1965 to 1973, and as its Chairman from 1969 to 1973. During this time, research on filariasis, which had been at a relatively low level, was particularly stimulated under the auspices of the Program.

Dr. Leon Jacobs

Chairman (1965–1969)

Dr. Jacobs served as Chairman of the U.S. Panel on Parasitic Diseases from its inception in 1965 until 1969. During that time, he was Chief of the Laboratory of Parasitic Diseases and Acting Scientific Director at the National Institute of Allergy and Infectious Diseases. He has maintained a strong commitment to international tropical disease research and has subsequently held positions as the Director of the Fogarty International Center at the National Institutes of Health as well as President and Chairman of the Board of the Gorgas Memorial Institute. His research interests have focused on toxoplasmosis, trichinosis, enterobiasis, and amebiasis, and he served the U.S. Army in the area of malaria control. Under Dr. Jacobs' chairmanship during the early development of the Program, it became clear that physiological, biochemical, and immunological studies on filariae and schistosomes, as well as studies on the natural history of these organisms, would be facilitated by provision for adequate supply of parasites and their vectors to the scientific community, and contracts for this purpose were initiated. From its inauguration, Dr. Jacobs has supported the opportunity that the U.S.-Japan Cooperative Medical Science Program offers for young scientists from both sides of the Pacific to become familiar with each other's work and interests.

JAPANESE PARASITIC DISEASES PANEL

Dr. Kazuo Yasuraoka

Chairman (1988–Present)

Since 1965, when the Program began, Dr. Yasuraoka has dedicated himself to it as a secretary (1965–1978) and a member of the Japanese Parasitic Diseases Panel (1979–1988). In the National Institute of Health, Tokyo, and in the Institute of Basic Medical Sciences, University of Tsukuba, he has continued to study the biology of hookworms and schistosomes, *in vitro* cultivation of parasitic helminths, and the control of snail hosts of schistosomiasis. In addition, Dr. Yasuraoka has served several times as a WHO Temporary Adviser in the control of schistosomiasis in Africa and Southeast Asia. He has been directing the WHO Collaborating Center for Chemical Control and Laboratory Culture of Amphibious Snail Hosts of *Schistosoma japonicum* at his Department of Medical Biology, University of Tsukuba. From 1972 to 1981, as a leader of Japanese experts he greatly contributed to the success of the Japan-Philippine Collaborative Research Program on the control of schistosomiasis in the Philippines. Since his appointment as Chairman of the Japanese Parasitic Diseases Panel in 1988, Dr. Yasuraoka has been very active in involving many younger scientists in parasitological research using newer technologies, particularly the use of monoclonal antibodies and recombinant DNA technologies.

Dr. Shigeo Hayashi

Chairman (1979–1988)

Since graduation from the School of Medicine at the University of Tokyo in 1947, Dr. Hayashi has been interested in helminthic diseases, especially in their epidemiology, and he has been highly successful in introducing statistical techniques into parasitology.

He trained many young medical scientists while he held the successive posts of Professor of the School of Medicine at Yokohama City University, Director of the Department of Parasitology, and Director-General at the National Institute of Health, Tokyo. While serving in these posts, he actively participated in the eradication of parasitic diseases as a member of several WHO Expert Committees, and strongly contributed to international medical cooperation.

From 1979 to 1988 he served as the Chairman of the Japanese Panel on Parasitic Diseases. One of his greatest achievements during his tenure was his ingenious coordination of many research projects by Japanese medical scientists, which contributed greatly to the Program's overall success.

Dr. Muneo Yokogawa

Chairman (1971–1979)

Dr. Yokogawa devoted himself to the Japanese Parasitic Diseases Panel as a member (1965–1971) and as Chairman (1971–1979). Under his leadership, the Panel worked on chemotherapy and pharmacology of antischistosomal and antifilarial drugs, biological and genetic control of vectors, and immunoserology, with special emphasis on schistosomiasis control in endemic areas in Japan. This work resulted in complete eradication of the disease from the last endemic region, the Kofu Basin; no new cases have been reported there since 1977.

On the other hand, Dr. Yokogawa was instrumental in inviting and encouraging young scientists as well as senior investigators to attend local meetings and joint conferences so as to stimulate interest in a broad spectrum of scientific fields related to schistosomiasis and filarasis. His efforts bore fruit and many of the young scientists, especially immunologists, joined in activities of the Japanese Panel.

Dr. Yokogawa worked closely with the Chairmen of the U.S. Parasitic Disease Panel, which facilitated scientific interaction of investigators attending the Joint Conferences and the sending of young scientists to U.S. laboratories.

Dr. Manabu Sasa

Chairman (1968–1971)

Dr. Sasa has been one of the most outstanding figures in the field of medical science in Japan. He was involved in the Japan-U.S. Cooperative Medical Science Program since its beginnings in 1965. His dedication to the Program was concentrated in the direction of the activities of excellent researchers from both countries in the study of specially selected targets. While he served as the Panel member (1965–1968) and later as the Chairman of the Japanese Panel on Parasitic Diseases (1968–1971), he concentrated all his efforts only on the two major human parasitic diseases; namely, schistosomiasis and filariasis.

Knowledge of both diseases either in basic research or in applied fields was strikingly enriched. It has contributed to the successful control of these diseases in many parts of the world. Dr. Sasa was a member of Japanese Delegation from 1974 to 1988, during which time, Panel guidelines were expanded to include malaria and other hemoprotozoan infections. His leadership was effective and essential in improving and updating the Program.

Dr. Yoshitaka Komiya

Chairman (1965–1968)

Dr. Komiya graduated from the School of Medicine at the University of Tokyo in 1925 and as a result of his studies on trematodes, later published an excellent scientific work entitled, "The excretory system of digenetic trematodes."

He successively held the posts of Professor of the School of Medicine at Gunma University, Director of the Department of Parasitology, and Director-General at the National Institute of Health, Tokyo. He eagerly studied important problems of parasitic diseases in addition to guiding many younger scientists and playing a leadership role in the Japanese Society of Parasitology.

Dr. Komiya worked energetically for the control of schistosomiasis as a member of the WHO Expert Committee on Schistosomiasis, and contributed globally to a reduction of damage caused by the parasite.

He was the first Chairman of the Japanese Panel on Parasitic Diseases, serving from 1965 to 1968, and as a member of the Japanese Delegation from 1968 to 1971. The Program greatly benefited from his efforts during its infancy. His service was distinguished, and his contribution was great.

GUIDELINES

PARASITIC DISEASES PANELS
United States-Japan Cooperative Medical Science Program

The Parasitic Diseases Panels have a primary interest in research on selected helminthic and protozoal diseases with special emphasis on vector-borne parasitic diseases such as, but not limited to, malaria, schistosomiasis, and filariasis. Sponsored studies share a long-term goal of alleviation of these diseases. Strategies include vector control, interruption of the parasite life cycle, and reduction of the amount and effects of infection in man by vaccination, chemoprophylaxis, or other modalities. Both basic and applied work is supported in the following areas:

Epidemiology;
Vector Biology and Control;
Parasite Biology;
Host-Parasite Interactions;
Pathology;
Immunology;
Biochemistry; and
Chemotherapy.

Special emphasis will be given to studies designed to explore the role of membrane chemistry and antigen differentiation in infection and immunity. In addition, the Panels are concerned with supply of parasites and infected animals to investigators and with availability of research workers in these fields.

U.S. Parasitic Diseases Panel

BROAD GOALS

From the inception of the U.S.-Japan Cooperative Medical Science Program in 1965 through the early 1980's, the focus of the Joint Panels on Parasitic Diseases was restricted to schistosomiasis and filariasis. The current status of these two groups of parasites as major causes of human suffering throughout the world's tropical and subtropical regions is generally little improved. In fact, the diseases are still spreading in many developing countries, and it is generally estimated that each disease afflicts over 250 million people. Although direct mortality from these infections is low, their profound effect upon human health and well-being and their interaction with malnutrition and other parasites and microbial agents contributes to substantial morbidity and places a significant burden on health services in developing countries where they occur. The importance of these two diseases was underscored when, in 1976, WHO included schistosomiasis and filariasis as two of the six diseases for selected study in their Special Programme for Research and Training in Tropical Diseases.

The original 1965 guidelines directed the Panels on Parasitic Diseases to focus their activities on the two important vector-borne human helminthiases, schistosomiasis, and filariasis. This rather limited focus was reaffirmed in 1975 when the guidelines were reviewed by the Panels. A wide variety of studies, involving both basic and applied research on these two diseases, were continued. The success of this Program over the two decades of the Cooperative Medical Science Program is clearly demonstrated by growth in research publications and the appearance of many new investigators pursuing relevant research.

However, by the early 1980's it became apparent that application of some of the newer research technologies in immunology and molecular biology, employed to study blood and tissue protozoan infections, such as malaria, leishmaniasis, and trypanosomiasis, had considerable relevance for research in schistosomiasis and filariasis. The Panels noted the similar investigative problems between many helminthic and protozoal diseases including vector interactions, pathogenesis, immunologic consideration, and other factors. These considerations resulted in changing guidelines to include malaria and other vector-borne parasitic diseases. The new guidelines have been presented at the beginning of this section.

Appropriately, the focus of the Parasitic Diseases Panels is to promote, encourage, and abet cooperative international research on not only filariasis and schistosomiasis but malaria and other vector-borne parasitic diseases. The complex biology of the parasites involved and of their pathologic effects on humans presume a long-term commitment to develop an understanding of the disease processes and formulating specific means for their control. Complexities of their cycles involve molluscan and arthropod vectors, as well as human and other mammalian hosts. This necessitates a multidisciplinary approach to all facets of their life cycle in order to find the weak links that scientists who work in specific and related fields might exploit. Such disciplines as parasitology, malacology, entomology, genetics, ecology, biochemistry, pharmacology, immunology, molecular biology, and other basic disciplines must all interact, in the laboratory and in the field, to achieve success in combating these parasites.

PROGRESS IN PRIORITY AREAS

Schistosomiasis

A particularly broad range of investigation in schistosomiasis has been carried out and includes research in multiple areas including immunology, pathogenesis, pathophysiology, biochemistry, and chemotherapy. The following early research accomplishments on schistosomiasis should be highlighted: (1) demonstration of protective immunity in primates against *Schistosoma japonicum* by exposure to homologous, x-irradiated cercariae, (2) demonstration of the relationship between cell-mediated immunity and schistosome granuloma formation, (3) definition of the lipid metabolism of schistosomes, and of their dependence on an exogenous supply of cholesterol, (4) elaboration of the conformational requirements for schistosomicidal activity of nitroheterocyclic drugs, and (5) establishment of new experimental models of *Schistosoma haematobium* infection in subhuman primates, with pathologic reactions similar to that of humans.

A wide variety of animal models was used for schistosome research with expanded use of inbred strains of mice and utilization of hamsters, rabbits, and primates. This led to significant advances and a better understanding of many features of schistosome infections which included mechanisms of protection and immunoregulation and the chronology of the pathologic response. In studies of *Schistosoma japonicum*, the Philippine strain of the parasite was consistently more pathogenic than those of China, Taiwan, or Japan. *S. mekongi* is highly pathogenic for hamsters and mice but dogs, the natural reservoir, suffer only minor pathologic changes and tend to self-cure over time. The baboon is the best animal host for study of pathologic changes due to *S. haematobium* infection.

Several investigators have studied immunity against challenge infection of *S. mansoni* cercariae after initial infection or previous exposure to highly irradiated cercariae. With irradiated cercariae it appears that development of protection correlates with delayed type hypersensitivity to schistosomal antigens, but not with antigen-induced blastogenesis or anti-schistosomal antibody responses. In baboons, the use of irradiated cercariae results in a reduction in the size of hepatic and intestinal egg-focused granulomas.

It was shown that eosinophils cause a small but significant antibody-dependent toxic effect on culture-derived schistosomula. Evidence suggests that activated macrophages may play an important role in rejection of schistosomula and that macrophage activation may be a major determinant factor of the host immune status. Through use of various model systems it was shown that inbred mice strains vary widely in their protection due either to chronic primary infection or irradiated-cercarial immunization.

Major early advances in schistosomiasis also include the sequential monitoring of the immunopathology of cercaria-induced infections by modern quantitative, immunological, and pathophysiological techniques. The role of cell-mediated immunity in granuloma formation was best demonstrated by use of guinea pigs and chickens that are normally unsusceptible to schistosomiasis. The immunological mechanisms activated by schistosome eggs vary among parasite species and granulomatous responses are subject to eliciting and modulating influences. This has considerable significance since modulation of the host cell response to schistosomes could have therapeutic value in human infection, particularly in the acute, severe, "toxemic" stage.

The use of murine models of schistosomiasis has allowed extensive study of the pathophysiology of these infections. Cell-mediated immune mechanisms are central to schistosome egg-induced granuloma formation, and a variety of immunoregulatory circuitries are known to then modulate this granuloma formation. Immunological studies of human schistosomiasis have demonstrated that several regulatory mechanisms develop as chronic human infections are established. These include antigen-specific and non-specific humoral regulation, antigen-induced suppressor T cells, antigen-specific adherent cell-dependent suppression, histamine-receptor bearing T suppressor cells, and anti-idiotypic T cell responses during infection.

Immunopathogenic studies showed the dependence on T-lymphocytes for schistosome ova granuloma formation. Differences exist in biologic and host immune responsiveness to infections with *S. mansoni* and *S. japonicum*, which suggests possible differences in egg antigens between the species. *In vitro* systems were developed in tissue culture which showed the formation of actual granulomas around eggs.

The complexity of cell interactions that take place during the chronic inflammatory process of schistosomiasis was studied by several investigators. In addition to lymphocytes, macrophage and mast cell function in an accessory regulatory role in the inflammatory and reparatory process in the schistosome egg granuloma was examined. Development of schistosome egg antigen-specific T cell clones was a major advance in the continued evaluation of the immunopathogenesis and immunoregulation of *S. mansoni* egg-induced granulomas. These clones respond specifically to schistosome egg antigen by undergoing blastogenesis, by producing local delayed type hypersensitivity reactions, by producing various lymphokines, and initiating the generation of granulomas *in vitro*. The role of the granulomatous inflammation elicited by schistosome eggs in hepatic fibrogenesis is being examined. Findings provide strong circumstantial evidence that hepatic fibrosis, vascular smooth muscle hyperplasia, and endothelial proliferation results from biologically active molecules elaborated by cells in the granulomas. Not only are these studies relevant for schistosomiasis, but they provide a model that may explain the pathogenesis of tissue fibrosis in other diseases associated with chronic inflammation, including lymphatic filariasis.

In *Schistosoma japonicum*, granulomatous reactivity and immunoregulation demonstrably involves both cellular and humoral mechanisms. It has been shown that idiotypic and anti-idiotypic regulation are both important at given times after murine infection. Anti-soluble egg antigen (SEA) MAbs can suppress granuloma formation, as can anti-anti-SEA MAbs. The identification of a dominant, cross-reactive idiotype which is prominently involved in this regulation is a major step forward in delineating the steps in this process.

Low responsiveness in egg granuloma formation in the liver of mice was induced by different immunization schedules using eggs. Low responsiveness of C57BL/6 mice in protective immunity against *S. japonicum* infection was demonstrated by using a vaccine model. Epitope specific regulatory mechanisms may exist in *S. japonicum* soluble egg antigen-driven T lymphocyte responses in man.

Extensive progress has been made in purification of schistosome antigens and in cloning DNA sequences that encode for various schistosome antigens. Some of the newly characterized antigens bear potential as diagnostic reagents to monitor effective chemotherapy, while others detect very recent infections. Successful immunization schemes utilizing purified antigens and anti-idiotypic monoclonal antibodies have been reported, and a variety of studies indicate a central role for activated macrophages in immunization-induced protection.

Much work was related to the potential development of a schistosome vaccine. The purification and identification of antigens, particularly those at the egg stage, were notable advances. From these studies hybridoma technology was used for development of monoclonal anti-soluble egg antigen component antibodies. This led to development of a very sensitive radioimmunoassay procedure for diagnosing of *S. mansoni* infection.

A purified, cloned, and expressed molecule identified as schistosome paramyosin (97 kD) is one of the major, but not sole, immunogens in crude cercarial preparation. T lymphocyte sensitization is essential for this form of immunization to be protective; protection occurs within 2 weeks of immunization and lasts at least 8 weeks after the last boost. The protective mechanism appears to operate within the skin, or as the parasites migrate from the skin to the lungs. Mouse strains have been identified which do or do not become resistant under this regimen, and their use may facilitate identification of the mechanisms involved.

Experiments utilizing preparations of the cercarial glycocalyx in inducing protective immunity against a primary schistosome infection suggest that the immunization may induce suppression or tolerance. cDNA clones encoding schistosome superoxide dismutase are being examined to explore extent of sequence homology of this potential target enzyme. A cDNA clone encoding schistosome tropomyosin has been identified that shows homology to various species.

Studies on the occurrence of hepatocellular carcinoma in relation to schistosomiasis have been carried out. In a group of mice exposed to *S. japonicum*, and provided with a carcinogenic diet containing 0.02% Trp–p–2, hepatocellular carcinoma was found in 9 out of 13 mice after 57 weeks postinfection. In another line of experiments the presence of Ha-*ras* related sequences was found in the DNA of schistosome. Amplified Ha-*ras* sequence was detected in the DNA of a hepatoma caused by chronic infection with *S. japonicum*.

The roles of snail hemocytes in resistance against schistosome sporocysts are being studied, and it is clear that hemocytes react against specific sporocyst surface molecules. These molecules are now defined immunologically by monoclonal antibodies (MAbs) which can reduce hemocyte-mediated killing of sporocysts by over half. Furthermore, hemocytes from genetically resistant snail strains appear to have a higher basal rate of metabolism and phagocytosis than those from susceptible strains.

The need for better antischistosomal drugs remained a high priority for research. Despite considerable progress in cultivation of schistosome life cycle stages, a major gap existed in the inability to promote and maintain reproductive activity under axenic conditions. This has considerable relevance in development of antischistosomal drugs. Finally, the development of molluscan cell culture lines made new research opportunities possible such as evaluation of defense mechanisms in molluscs.

Potential problems associated with the mutagenic, teratogenic, and carcinogenic potential of some antischistosomal compounds are significant. This led to numerous studies on analogs of effective compounds and development of drug resistance. Substantial progress in cultivation of schistosome larval stages was made, but completion of the complete life cycle was not accomplished. A major development was the successful establishment of a cell line from *Biomphalaria glabrata* embryos.

Numerous studies were directed to isolation and characterization of schistosome proteins and proteolytic enzymes. Proteolytic enzymes of *S. mansoni* eggs are being studied to determine their role in the movement of eggs through tissues and their stimulation of host immune responses and granuloma formation. A large number of hybridoma cell lines were cloned which produce monoclonal antibodies and recognize a wide variety of epitopes from *S. mansoni* and *S. japonicum* egg antigens. Testing of some monoclonals in enzyme-linked immunosorbent assays (ELISA) indicated the potential yield of highly specific and sensitive immunodiagnostic procedures. Other studies identified the species-specific and gender-specific proteins of *S. mansoni*, *S. japonicum*, and *S. haematobium*. The immunoreactivity of these proteins was characterized by use of sera of infected hosts and with monoclonal antibodies. These studies should help develop probes (purified schistosomal proteins and monoclonal antibodies) useful for identification of the antigen and antigen/antibody complexes associated with granulomas and glomerulonephritis in infected mice.

Pharmacologic differences between longitudinal and circular muscles of schistosomes were examined which may be useful as targets for antischistosomal drugs. The calcium regulatory protein, calmodulin, is important in schistosomes. Inhibition of its action by potential chemotherapeutic agents may suggest new agents for drug therapy.

Schistosoma mansoni possesses the enzyme hypoxanthine guanine phosphoribosyltransferase (HGPRTase) for the salvage of purines, but lacks enzymes for *de novo* purine nucleotide synthesis. Studies have focused on the delineation of schistosome HGPRTase in hopes of finding differences between it and that of the host that might be exploited in drug development. Using a nick-translated plasmid containing the complete coding sequence for mouse HGPRTase, *S. mansoni* genomic DNA was restricted and resolved by Southern blots. Complementary sequences were found in a single Pst I fragment of approximately 1,400 base pairs. The same cDNA probe was used to screen a lambda gt11 *S. mansoni* cDNA library and two clones have been identified and purified to homogeneity. After subcloning and partial sequencing, the deduced amino acid sequence was 66% homologous with the sequence for mouse HGPRTase.

Mevinolin, a specific and potent inhibitor of HMG-CoA reductase, has been shown to inhibit egg production both *in vitro* and *in vivo*. Adult *Schistosoma mansoni* contains at least five forms of glutathione (GSH) S-transferase (SmGST). At least two members of the SmGST-1/2/3 family are coded for by unique mRNAs. Studies on excretory end-products of adult *S. mansoni* suggested a partial reversed TCA cycle is active under anaerobic conditions.

The major secreted protease of schistosome cercariae is a serine protease released from acetabular glands in response to skin lipid. A three dimensional model of the cercarial proteinase is being utilized to obtain specific information about the substrate binding specificity of the enzyme's active site.

Filariasis

In filariasis research, a new small laboratory animal model of lymphatic filariasis was established through infection of the jird, *Meriones unguiculatus*, with *Brugia pahangi* and *B. malayi*. This model generated many new advances in our understanding of lymphatic filarial infections. Quantitative studies on pathogenesis of *Brugia* infections in cats were initiated. Efforts to improve filarial serodiagnosis were instructive in detecting extensive cross-reactivity between filarial and other helminth antigens. Field studies were reported on the vector mosquito of the *Aedes scutellaris* group in the Pacific as were biochemical studies on the bacteria-free rearing of *Aedes* mosquitoes which defined the host's requirements of RNA and of zinc.

It was shown for the first time that the human species, *Wuchereria bancrofti*, matured, though microfilaremias did not develop, in macaque monkeys. Studies on the pathogenesis of *Brugia malayi* and *B. pahangi* infections in dogs and cats gave new insight into the underlying mechanisms of human pathologic changes caused by these lymphatic filariae.

The importance of the Mongolian jird, *Meriones unguiculatus*, as a laboratory host for the lymphatic filariae was confirmed and extended. A significant finding was that intraperitoneal inoculation of infective larvae resulted in development of adult worms and production of microfilariae in the body cavity. This facilitated recovery of all stages of the parasite for use in experimental procedures, the collection of material for use as antigen in serological and immunological studies, and development of biochemical studies on these parasites. The *Dirofilaria* dog model and the various *Brugia* models showed that separate immunological mechanisms were operative in the suppression of microfilaremia versus protection from reinfection.

A significant new vector development was the demonstration that cytoplasmic incompatibility in *Culex* related to the presence of a rickettsia-like organism, *Wolbachia pipientis*, which could have considerable significance in vector control.

A major advance in filariasis research was production of experimental patent infections of *Wuchereria bancrofti* in Taiwan macaques. Microfilaremias were low and transient yet this accomplishment was the first definitive indication for the eventual establishment of a primate model for the study of classical *W. bancrofti* infection. This parasite is the major cause of human filarial elephantiasis. Jirds and other rodents were widely exploited for pathologic and immunologic studies. Concurrent feline infections with *Brugia malayi* and streptococci resulted in tissue inflammation and edema consistent with that of chronic human infections. In addition to the lymphatic filariae, it was demonstrated that the African eye worm, *Loa loa*, could be maintained routinely in baboons and several species of monkeys; deerflies from the southeastern United States can serve as efficient experimental vectors. Use of the Mongolian jird-*Brugia* model for study of the immune response in filariasis provided evidence for the role of cell-mediated immunity in these infections. Filarial infections induced suppressor cell activity that inhibited lymphocyte proliferation *in vitro*. Increased levels of circulating immune complexes were noted in the sera of jirds infected with *B. pahangi*, but there was no correlation between immune complex levels and the numbers of circulating microfilariae.

Experiments utilizing *Brugia* in jirds showed that the pathologic response to parasite antigen is granulomatous, causing thrombosis of lymph vessels. The basis for this response is a specific cell-mediated immune response. Parallel studies demonstrated, however, that even in nude mice, parasites can cause profound changes in cells lining lymphatics.

Studies were pursued on the biochemistry of larval and adult filariae, with a view toward developing better chemotherapeutic agents. Pyruvate metabolism was evaluated in *B. pahangi* and *Dipetalonema viteae*. The tricarboxylic acid cycle pathway is regarded of doubtful importance as an energy-yielding pathway in these parasites.

Through Panel-sponsored contract research short- and long-term cryopreservation of microfilariae and infective-stage filarial larvae of sheathed and unsheathed species was accomplished. This provided opportunities for the transport of rare or scarce materials to laboratories capable of working with various aspects of these parasites. Work with *W. bancrofti* and *Mansonella ozzardi* could be enhanced considerably. Studies on vector biology showed the usefulness of American species of deerflies for transmission of *Loa* infection and that local species of blackflies, *Simulium* spp., were not infected with *Onchocerca volvulus*. This was due to the presence of teeth in the mouthparts of these flies which damage ingested microfilariae.

Vector studies in Guatemala, Panama, and Colombia showed that there is a potential hazard for extension of onchocerciasis and mansonellosis in new geographic areas due to exposure of greater numbers of persons through new road or land development projects. Fortunately, new rearing and maintenance techniques and renewed interest in vector-parasite interactions resulted in a resurgence of vector studies in filariasis, leishmaniasis, and other arthropod-transmitted diseases. This work includes biochemical genetics and evaluation of cell responses and cellular defense mechanisms in both filaria-susceptible and filaria-resistant mosquitoes. In addition to mosquito and blackfly susceptibility, culicoid susceptibility is being examined with use of isoenzyme analysis and other techniques.

Studies of *Dirofilaria immitis* and its development in species of *Aedes* and *Anopheles* have focused on the physiological, ultrastructural, and biochemical analyses of factors in the insect host cell which determine susceptibility to infection and the effects of infection on the Malpighian tubules of the mosquito. The transport physiology of the infected tubules is markedly affected in *Aedes* but not in *Anopheles* which has significance with respect to transmission potentials. There may be broader applications since a detailed ultrastructure study of mosquito osmoregulatory organs is being conducted. Relevant findings may indicate the need to evaluate the ecology of various mosquito species. Whether microfilariae can develop within a mosquito may be determined by the ability of microfilariae to avoid and/or inhibit the vector's immune response. Possibly, microfilariae avoids the vector reaction by being coated with host antigens during midgut penetration in appropriate vectors. The peritrophic membrane appears not to function as a barrier to migration of microfilariae out of the mosquito stomach. In a related study, it was shown that the peritrophic membrane and midgut epithelium of culicoids are not barriers to *Dipetalonema* and *Mansonella* microfilarial migration out of the midgut.

In the mosquito-filarid relationship, it has now been shown through transplantation studies that the refractoriness of some *Aedes aegypti* strains for the development of *Dirofilaria immitis* microfilariae is a consequence of the environment within their Malpighian tubules. The arrest of microfilarial development in the refractory strain occurs soon after their entry into the Malpighian tubules. Encapsulation of microfilariae in this system is due to the response of mosquito hemocytes and their protein-polyphenolization reactions. Certain hemocytes synthesize and release melanin, which then contributes to this biochemical process. Mosquitoes older than 14 days have few hemocytes and mount a much less effective anti-microfilarial response. Male mosquitoes have very few hemocytes and are almost totally "immunoincompetent." The activation of hemocyte monophenol oxidase upon infection by microfilariae appears to parallel the degree of resistance expressed by a given species of mosquito.

Microfilariae form specific cyclooxygenase pathway derived prostaglandins, including prostaglandin E_2 and 6-keto-prostaglandin $F_{1\alpha}$, that could be pertinent mediators of interactions between parasites and various immunologic effector cells and platelets.

Continued field trials of the efficacy of ivermectin for onchocerciasis have been very successful. Yearly single dose treatment convincingly leads to continued lower skin microfilaremia. Importantly, treatment was also associated with a significant decrease in ocular disease manifestations. These results indicate that ivermectin in a single oral dose only yearly results in a sustained reduction of parasite load and ocular disease, despite ongoing transmission.

Malaria

Although malaria research has been a component of the U.S.-Japan Program during only this 5-year period, this area has brought a vigorous scientific enterprise to join the previous efforts in schistosomiasis and filariasis. This is particularly gratifying in view of the increasingly serious and uncontrolled nature of the global malaria problem.

The major erythrocyte receptor for *P. falciparum* is the family of sialoglycoproteins known as glycophorins, the terminal sialic residues being a critical site in that interaction. A major merozoite surface antigen, Pf200, or Mr 200,000, binds to the sialic acids of glycophorin, suggesting it is involved in host cell recognition. The functionally important regions of this polypeptide are now being studied.

Three proteinases of *P. falciparum* have been characterized whose activities were expressed stage-specifically in the life cycle of the erythrocytic parasite. Trophozoites had an Mr 28,000 proteinase that was inhibited by inhibitors of cysteine proteinases. Mature schizonts had an Mr 35–40,000 proteinase that was also inhibited by cysteine proteinase inhibitors. Merozoite fractions had an Mr 75,000 proteinase that was inhibited by serine proteinase inhibitors. The stage-specific activities and inhibitor sensitivities of these proteinases suggested potential functions for each enzyme. A cysteine proteinase has a critically important role in hemoglobin degradation in the food vacuole of trophozoites. The Mr 28,000 trophozoite proteinase has been identified as being similar to the mammalian lysosomal proteinase cathepsin L, which supports its potential role as a food vacuole enzyme. Highly specific peptide inhibitors of cathepsin L inhibit hemoglobin degradation within the food vacuole and block the multiplication of *P. falciparum* at concentrations well below those which are toxic to mammalian cells *in vitro* or mice *in vivo*. Thus, this indicates that malaria parasites are more sensitive to inhibitors of lysosomal proteolysis than are mammalian cells and suggests an approach to antimalarial chemotherapy that takes advantage of this difference between the parasite and its host.

Steps in the invasions process subsequent to receptor binding are poorly defined. Electron microscopic studies show that following parasite binding, the erythrocyte membrane invaginates and forms a vacuole which eventually surrounds the invading parasite. The parasite factors which trigger this radical deformation of the erythrocyte membrane are not known although it has been suggested, based on electron microscopic studies, that contents secreted from the rhoptry organelle could be responsible. A marked dephosphorylation of host cell membrane proteins accompanies merozoite invasion. The rhoptry organelle and its contents are being characterized with the intention to define the role of its components in merozoite invasion. These studies have taken two different approaches. One involves a monoclonal antibody to a rhoptry protein of 110k, which coprecipitates a Mr 140k and 130k rhoptry protein. The 110k rhoptry protein is secreted into the erythrocyte membrane during merozoite invasion and persists in the membrane for up to 24 hours. Conveniently, the 140k–110k protein complex also binds to mouse erythrocytes, cells that are invaded to a certain degree by *P. falciparum*. The properties of invasion of *P. falciparum* of erythrocytes correlate with the properties of binding of the rhoptry proteins to erythrocytes, suggesting the proteins play a role in invasion.

Studies on *Plasmodium falciparum* merozoite invasion into human erythrocytes has yielded important novel information with respect to the membranes in facilitating the invasion process. The highly complex series of biochemical membrane events can be divided into three major categories that appear equally important in facilitating the parasites' survival in the host cell: (I) Formation of the parasitophorous vacuolar membrane (PVM) from parasite phospholipids (PL) and cholesterol and from host cell membrane components, (II) the modulation of functional and structural properties of the host cell plasma membrane leading to biochemical and physical changes, and (III) apparent continued exchange between the erythrocyte and parasite membrane systems with significant redistribution of major host cell membrane components.

The plasmodial parasite selectively accumulates PL in the rhoptry organelles. These PL are transferred to the host cell membrane at the site of attachment and contribute to the formation of the vacuolar membrane. While evidence exists for cholesterol transfer at the same time, this essential membrane component does not exhibit the same redistribution as found for PL. At the same time, there is evidence from digitized video-intensified fluorescence microscopy (DVIFM) using fluorescent PL derivatives that parasite PL diffuse into the host cell membrane. Since standard biochemical analysis did not yield significant changes in the lipid composition of membranes from parasitized erythrocytes, Raman spectroscopy was used to study the results of PL partitioning into the host cell membrane. The results have been obtained on schizont-infected human erythrocytes. Dramatic differences in the temperature-dependent phase transitions of the erythrocyte plasma membrane occur at the time of invasion and are maintained throughout the intraerythrocytic parasite maturation. The main lipid-mediated phase transition (I_{2940}/I_{2850}) in plasma membranes changes from 41 °C in normal erythrocytes to 2 to 7 °C in parasitized cells. These changes are associated with major conformational rearrangements of membrane proteins that are also supported by a novel computer-modeled analysis of membrane protein conformation based Raman spectra of standard proteins of known structure. The analyses have yielded changes of more than 30% of both the random coil and alpha-helical structure. As the proteins undergo this rearrangement the lipid-protein interactions are reduced, which facilitates major modifications of the lipid phase of the plasma membrane.

The major intrinsic membrane proteins band 3 and glycophorin are transferred into parasitophorous vacuolar membrane (PVM) during the invasion process. As band 3 accounts for over 75% of the lipid-protein interactions in the erythrocyte plasma membrane, the reduction of the lipid-protein interactions in the erythrocyte plasma membrane has to be due to a reduction of band 3 molecules or marked conformational rearrangement of the protein. The latter seems most likely as immunochemical quantitation of band 3 using DVIFM do suggest no quantitative loss of band 3 in the host cell membrane at the time of invasion. Such a loss, linked to an absolute gain of band 3 in the PVM, is seen later during parasite maturation.

Infections with the human malaria *Plasmodium falciparum* are characterized by the attachment of parasitized erythrocytes to the endothelial cells that line the capillaries. Cytoadherence has a critically important role in the pathogenesis of falciparum malaria since it permits the parasites to evade spleen-dependent immune mechanisms and since the sequestered parasites may occlude blood flow, as seen in cerebral malaria. The biochemical mechanisms responsible for erythrocyte adherence to the endothelial cell are still undefined. A monoclonal antibody to knob-bearing erythrocytes infected with the FCR-3 strain of *P. falciparum*, designated 4A3, is an IgM that reacts (by means of immunofluorescence) with the surface of unfixed erythrocytes bearing mature parasites of the knobby line; it does not react with knobless lines or uninfected erythrocytes. By immunoelectron microscopy the monoclonal antibody 4A3 was localized to the knob region. In an *in vitro* cytoadherence assay, the monoclonal antibody partially blocked the binding of knob-bearing cells (FCR-3 strain) to formalin-fixed amelanotic melanoma cells. The monoclonal antibody was used to immunoprecipitate a protein from extracts of knobby erythrocytes that had been previously surface iodinated. By a two-dimensional peptide mapping technique, the antigen recognized by the monoclonal antibody was found to be structurally related to band 3 protein, the human erythrocyte anion transporter.

Three structural domains of knob proteins of *P. brazilianum* have been expressed in *E. coli* and purification procedures for the expressed proteins established. The purified products (~95% purity) have been tested for reactivity with immune human sera from different geographical areas as well as for reactivity with human erythrocyte membranes. The most significant results of these studies is that the central lysine-rich domain of KP is the most conserved and immunogenic fragment. Furthermore, this same fragment is the only one which shows specific and high affinity binding to inside-out red cell membrane vesicles. Thus, it appears that the functional domain of KP is also highly immunogenic in man.

To investigate the molecular basis of cytoadherence with infected human erythrocytes, *in vitro* techniques were developed for modulating the cytoadherence phenotype of cloned, culture-adapted parasites. C32 melanoma cells, which also bind infected erythrocytes, were used for these studies. In initial experiments, it was found that culture-adapted parasites were poorly cytoadherent (compared with parasites from humans or monkeys) or noncytoadherent. To select for cytoadherent (C+) parasites, infected erythrocytes containing knobbed (K+) parasites were incubated with monolayers of C32 melanoma cells, the nonadherent cells removed by washing, and the remaining adherent cells cultured. To select for noncytoadherent (C−) parasites, the nonadherent cells were cultured. Using this technique, a large (10–15 fold) increase in cytoadherence with the ITG2F6 clone of *P. falciparum* was seen and a sequence of reversible changes in the cytoadherence phenotype of cloned parasites between populations with increased cytoadherence and decreased cytoadherence occurred.

Using cytoadherent parasites prepared as described above a family of large (Mr>240,000) variable proteins on the surface of infected erythrocytes was identified. The members of this protein family were cleaved by the same concentration of trypsin that prevented cytoadherence and had several biochemical properties in common with the strain-specific proteins on infected erythrocytes from Aotus monkeys. Using ITG2F6 parasites with different cytoadherence phenotypes, it was found that the expression and variation of the members of this protein family was correlated with the cytoadherence phenotype of the parasites. The protein was not detected with uninfected erythrocytes, and noncytoadherent parasites did not have the protein on the surface of infected erythrocytes. Parasites with increased cytoadherence had larger proteins (Mr 260,000–320,000) and parasites with decreased cytoadherence had smaller proteins (Mr 240,000). The strong correlation between the cytoadherence phenotype of the parasites and the expression of the members of this protein family is consistent with their having a role in mediating cytoadherence. The correlation of increased or decreased cytoadherence with expression of larger or smaller forms of the protein, respectively, suggests that the smaller proteins lack domains that mediate or promote cytoadherence. In contrast to these results, no change in expression and no variation in the molecular size of mature erythrocyte surface antigen (MESA) or knob-associated histidine-rich protein (KAHRP), two other malarial proteins that have been localized to the membrane of infected erythrocytes was observed.

The family of proteins described above could potentially be of parasite origin or, alternatively, they could be altered host cell proteins. These two possibilities were investigated in three ways. **First**, re-expression of both cytoadherence and the cytoadherence protein after trypsin treatment of infected erythrocytes was prevented by incubating the infected erythrocytes with cycloheximide, whereas controls incubated without cycloheximide re-expressed both cytoadherence and the protein. Thus, parasite protein synthesis was required for re-expression of both the cytoadherent phenotype and the cytoadherence protein. **Second**, three proteolytic cleavage fragments of the cytoadherence protein co-migrated on the one-dimensional SDS gel with metabolically labeled fragments, indicating that the cytoadherence protein was newly synthesized and therefore, of parasite origin. **Third**, an immunoprecipitation analysis of the cytoadherence protein using monospecific rabbit anti-sera against erythrocyte membrane proteins (Band 3, glycophorin, spectrin, 4.1) and human immune sera containing antibody to the cytoadherence protein was performed. This demonstrated the following: **1.** The cytoadherence protein is the principal protein antigen on infected erythrocytes that is recognized by antibody in human immune sera. **2.** There is no immunological cross-reactivity between the cytoadherence protein and the host erythrocyte proteins. Taken together, these results indicate that the members of the cytoadherence protein family are of parasite origin and are not altered host proteins.

Recently, it has become apparent that immunity to malaria is likely to involve the responses of both B cells (antibody) and T cells (in the form of T cell help and cell-mediated responses). In the *P. chabaudi adami*-mouse system it is known that T lymphocytes from infected mice, antigen-specific T cell lines, and now such T cell clones can transfer protection against challenge infection to naive mice. The protective T cell clone is CD4+, and produces IFN-gamma and IL-2. A new finding indicates that intravenous injection of solubilized *P. chabaudi adami* antigen leads to the development of transferable CD8+ antigen-specific suppressor cells that are active in decreasing specific delayed-type hypersensitivity reactions, but do not have an overt effect on the outcome of infection. Studies to define the antigens to which these effector and suppressor T cells respond are in progress using cloned or purified plasmodial antigens.

Macrophages activated by lymphokines or recombinant gamma-IFN are able to kill intraerythrocytic *P. yoelii* malaria parasites. The possibility that gamma-IFN might have an effect on the course of the erythrocytic phase of rodent malaria *in vivo* was investigated. About ten thousand units of recombinant rodent gamma-IFN/day had a dose-dependent protective effect on SW, Balb/c ByJ, and CBA mice infected with the lethal variant of *P. yoelii 17X* PyL. There was a greater effect of gamma-IFN on Balb/c ByJ mice that are more susceptible to malaria than on the CBA/J mice that are more resistant. There was little effect of gamma-IFN on mice infected with *P. yoelii* NL (nonlethal) except in Balb/c ByJ.

Because Balb/c ByJ mice responded differently *in vivo* to exogenously administered gamma-IFN, the endogenous gamma-IFN response in these mouse strains during infection with PyL and PyNL was investigated. In Balb/c ByJ mice infected with Py NL there was an early burst of gamma-IFN by spleen cells in response to soluble malaria antigens. As parasitemia increased, however, gamma-IFN levels declined to control levels. In contrast, in the same strain of mice infected with PyL, there was no increased gamma-IFN response throughout infection. In CBA/J mice, there was an initial burst of gamma-IFN by spleen cells from mice infected with both PyL and PyNL. Similar to the findings in Balb/c ByJ mice, gamma-IFN levels declined as parasitemia increased. However, as the infection resolved, animals infected with both L and NL parasites produced a second burst of the lymphokine. The H_2O_2 responses of splenic cells were followed simultaneously with gamma-IFN responses. A similar pattern of response was observed, consistent with the fact that gamma-IFN activates macrophages for increased secretion of H_2O_2. These results suggest that nonlethal malaria is able to induce a more effective gamma-IFN response and, perhaps, animals control the infection by activating macrophages. In contrast, mice infected with lethal parasites can only partially control the infection upon injection of exogenous gamma-IFN. Future studies will address the mechanisms of the protective response in nonlethal versus lethal malaria.

SHORT SUMMARY OF THE FIRST FIVE YEARS

A broad range of investigation on filariasis and schistosomiasis included immunology, pathogenesis, pathophysiology, biochemistry, and chemotherapy. The following research accomplishments were highlighted on schistosomiasis: (1) demonstration of protective immunity in primates against *Schistosoma japonicum* by exposure to homologous, x-irradiated cercariae; (2) demonstration of the relationship between cell-mediated immunity and schistosome granuloma formation; (3) definition of the lipid metabolism of schistosomes, and of their dependence on an exogenous supply of cholesterol; (4) elaboration of the conformational requirements for schistosomicidal activity of nitroheterocyclic drugs; and (5) establishment of new experimental models of *Schistosoma haematobium* infection in subhuman primates, with pathologic reactions similar to that of humans.

In filariasis research, a new small laboratory animal model of lymphatic filariasis was established through infection of the jird, *Meriones unguiculatus*, with *Brugia pahangi* and *B. malayi*. This model generated many new advances in our understanding of lymphatic filarial infections. Quantitative studies on pathogenesis of *Brugia* infections in cats were initiated. Efforts to improve filarial serodiagnosis were instructive in detection of extensive cross-reactivity between filarial and other helminth antigens. Field studies were reported on the vector mosquito of the *Aedes scutellaris* group in the Pacific as were biochemical studies on the bacteria-free rearing of *Aedes* mosquitoes which defined the host's requirements of RNA and of zinc.

SHORT SUMMARY OF THE SECOND FIVE YEARS

Research efforts were confined to schistosomiasis and filariasis; investigators working in schistosomiasis exceeded those in filariasis and the character of work was more sophisticated. The latter related specifically to the opportunity to employ defined laboratory animal models with shortened life cycles for schistosome parasites.

Major advances in schistosomiasis included the sequential monitoring of the immunopathology of cercaria-induced infections by modern quantitative immunological and pathophysiological techniques. The role of cell-mediated immunity in granuloma formation was best demonstrated by use of guinea pigs and chickens, which are normally unsusceptible to schistosomiasis. The immunological mechanisms activated by schistosome eggs vary among parasite species and granulomatous responses are subject to eliciting and modulating influences. This has considerable significance since modulation of the host cell response to schistosomes could have therapeutic value in human infection, particularly in the acute, severe, "toxemic" stage.

Problems associated with the mutagenic, teratogenic, and carcinogenic potential of antischistosomal compounds were significant. These led to numerous studies on analogs of effective compounds and development of drug resistance. Substantial progress in cultivation of schistosome larval stages was made; however, identification of the complete life cycle was not accomplished. A major development was the successful establishment of a cell line from *Biomphalaria glabrata* embryos.

During this period filariasis research was devoted principally to elaboration of model systems for the laboratory study of various filarial parasites. The classical, laboratory-maintained filariae—*Litomosoides carinii*, *Dipetalonema viteae*, and *Dirofilaria immitis* were used, but increased progress was made in studies on the lymphatic-dwelling filariae of the genera *Brugia* and *Wuchereria*. It showed for the first time that the human species, *Wuchereria bancrofti*, matured, though microfilaremias did not develop, in macaque monkeys. Studies on the pathogenesis of *Brugia malayi* and *B. pahangi* infections in dogs and cats gave new insight into the underlying mechanisms of human pathologic changes caused by these lymphatic filariae.

The importance of the Mongolian jird, *Meriones unguiculatus*, as a laboratory host for the lymphatic filariae was confirmed and extended. A significant finding was that intraperitoneal inoculation of infective larvae resulted in development of adult worms and production of microfilariae in the body cavity. This facilitated recovery of all stages of the parasite for use in experimental procedures, the collection of material for use as antigen in serological and immunological studies, and development of biochemical studies on these parasites. The *Dirofilaria* dog model and the various *Brugia* models showed that separate immunological mechanisms were operative in the suppression of microfilaremia versus protection from reinfection.

Despite increased study, no new antifilarial drugs were shown to be superior to diethylcarbamazine. A significant new vector development was the demonstration that cytoplasmic incompatibility in *Culex* related to the presence of a rickettsia-like organism, *Wohlbachia pipientis*, which could have considerable significance in vector control.

SHORT SUMMARY OF THE THIRD FIVE YEARS

A wide variety of animal models was used for schistosome research with expanded use of inbred strains of mice and utilization of hamsters, rabbits, and primates. This led to significant advances and a better understanding of many features of schistosome infections which included mechanisms of protection and immunoregulation and the chronology of the pathologic response. In studies of *Schistosoma japonicum*, the Philippine strain of the parasite was consistently more pathogenic than those of China, Taiwan, or Japan. *S. mekongi* is highly pathogenic for hamsters and mice but dogs, the natural reservoir, suffer only minor pathologic changes and tend to self-cure over time. The baboon is the best animal host for study of pathologic changes due to *S. haematobium* infection.

Immunopathogenic studies showed the requirement for T-lymphocytes in schistosome ova granuloma formation. Differences in biologic and host immune responsiveness to infections with *S. mansoni* and *S. japonicum* suggest possible differences in egg antigens between the species. *In vitro* systems were developed in tissue culture which showed the formation of actual granulomas around eggs.

Much work was related to the potential development of a schistosome vaccine. The purification and identification of antigens, particularly those at the egg stage, were notable advances. From these studies hybridoma technology was used for development of monoclonal anti-soluble egg antigen component antibodies. This led to development of a very sensitive radioimmunoassay procedure for the diagnosis of *S. mansoni* infection.

It was shown that eosinophils cause a small but significant antibody-dependent toxic effect on culture-derived schistosomula. Evidence suggests that activated macrophages may play an important role in rejection of schistosomula and that macrophage activation may be a major determinant factor of the host's immune status. Through various model systems it was shown that inbred mouse strains vary widely in their protection due either to chronic primary infection or irradiated-cercarial immunization.

The need for better antischistosomal drugs remained a high research priority. Despite considerable progress in cultivation of schistosome life cycle stages a major gap existed in the inability to promote and maintain reproductive activity under axenic conditions. This has considerable relevance in development of antischistosomal drugs. Finally, the development of molluscan cell culture lines made new research opportunities possible such as evaluation of defense mechanisms in molluscs.

A major advance in filariasis research was production of experimental patent infections of *Wuchereria bancrofti* in Taiwan macaques. Microfilaremias were low and transient, yet, this accomplishment was the first definitive indication for the eventual establishment of a primate model for the study of classical *W. bancrofti* infection. This parasite is the major cause of human filarial elephantiasis. Jirds and other rodents were widely exploited for pathologic and immunologic studies. Concurrent feline infections with *Brugia malayi* and streptococci resulted in tissue inflammation and edema consistent with that of chronic human infections. In addition to the lymphatic filariae, it was demonstrated that the African eye worm, *Loa loa*, could be maintained routinely in baboons and several species of monkeys; deerflies from the southeastern United States can serve as efficient experimental vectors. Use of the Mongolian jird-*Brugia* model for studying the immune response in filariasis provided evidence for the role of cell-mediated immunity in these infections. Filarial infections induced suppressor cell activity which inhibited lymphocyte proliferation *in vitro*. Increased levels of circulating immune complexes were noted in the sera of jirds infected with *B. pahangi*, but there was no correlation between immune complex levels and the numbers of circulating microfilariae. Studies were pursued on the biochemistry of larval and adult filariae, with a view toward developing better chemotherapeutic agents. Pyruvate metabolism was evaluated in *B. pahangi* and *Dipetalonema viteae*. The tricarboxylic acid cycle pathway is regarded of doubtful importance as an energy-yielding pathway in these parasites.

Through Panel-sponsored contract research, short- and long-term cryopreservation of microfilariae and infective-stage filarial larvae of sheathed and unsheathed species was accomplished. This provided opportunities for the transport of rare or scarce materials to laboratories capable of working with various aspects of these parasites. Work with *W. bancrofti* and *Mansonella ozzardi* could be enhanced considerably. Studies on vector biology showed the usefulness of American species of deerflies for transmission of *Loa* infection and that local species of blackflies, *Simulium* spp., were not infected with *Onchocerca volvulus*. This was due to the presence of teeth in the mouthparts of these flies which damage ingested microfilariae.

SHORT SUMMARY OF THE FOURTH FIVE YEARS

Several studies were directed to isolation and characterization of schistosome proteins and proteolytic enzymes. Proteolytic enzymes of *S. mansoni* eggs are being studied to determine their role in the movement of eggs through tissues and their stimulation of host immune responses and granuloma formation. A large number of hybridoma cell lines were cloned which produce monoclonal antibodies and recognize a wide variety of epitopes from *S. mansoni* and *S. japonicum* egg antigens. Testing of some monoclonals in enzyme-linked immunosorbent assays (ELISA) indicated the potential yield of highly specific and sensitive immunodiagnostic procedures. Other studies identified the species-specific and gender-specific proteins of *S. mansoni*, *S. japonicum*, and *S. haematobium*. The immunoreactivity of these proteins was characterized by use of sera of infected hosts and with monoclonal antibodies. These studies should help develop probes (purified schistosomal proteins and monoclonal antibodies) useful for identification of the antigen and antigen/antibody complexes associated with granulomas and glomerulnephritis in infected mice. Drug development for use against schistosomes is in progress. Pharmacologic differences between longitudinal and circular muscles of these parasites are being examined which may be useful as targets for antischistosomal drugs. The calcium regulatory protein, calmodulin, is important in schistosomes. Inhibition of its action by potential chemotherapeutic agents may suggest new agents for drug therapy.

The complexity of cell interactions that occur during the chronic inflammatory process of schistosomiasis was studied by several researchers. In addition to lymphocyte function, the role of macrophages and mast cells as accessory regulatory cells in the inflammatory and reparatory process in the schistosome egg granuloma was examined. Development of schistosome egg antigen-specific T cell clones was a major advance in the continued evaluation of the immunopathogenesis and immunoregulation of *S. mansoni* egg-induced granulomas. These clones respond specifically to schistosome egg antigen by undergoing blastogenesis, by producing local delayed type hypersensitivity reactions, by producing various lymphokines, and initiating the generation of granulomas *in vitro*. The role of the granulomatous inflammation elicited by schistosome eggs in hepatic fibrogenesis is being examined. Findings provide strong circumstantial evidence that hepatic fibrosis, vascular smooth muscle hyperplasia, and endothelial proliferation results from biologically active molecules elaborated by cells in the granulomas. Not only are these studies relevant for schistosomiasis, but they provide a model which may explain the pathogenesis of tissue fibrosis in other diseases associated with chronic inflammation, including lymphatic filariasis.

Several investigators have studied immunity against challenge infection of *S. mansoni* cercariae after initial infection or previous exposure to highly irradiated cercariae. With irradiated cercariae it appears that development of protection correlates with delayed type hypersensitivity to schistosomal antigens, but not with antigen-induced blastogenesis or anti-schistosomal antibody responses. In baboons, use of irradiated cercariae results in a reduction in the size of hepatic and intestinal egg-focused granulomas.

Vector studies in Guatemala, Panama, and Colombia showed that a potential hazard exists for extension of onchocerciasis and mansonellosis in new geographic areas due to exposure of greater numbers of persons through new road or land development projects. Fortunately, new rearing and maintenance techniques and renewed interest in vector-parasite interactions resulted in a resurgence of vector studies in filariasis, leishmaniasis, and other arthropod-transmitted diseases. This work includes biochemical genetics and evaluation of cell responses and cellular defense mechanisms in both filaria-susceptible and filaria-resistant mosquitoes. In addition to mosquito and blackfly susceptibility, culicoid susceptibility is being examined with use of isoenzyme analysis and other techniques.

Studies of *Dirofilaria immitis* and its development in species of Aedes and Anopheles have focused on the physiological, ultrastructural, and biochemical analyses of factors in the insect host cell which determine susceptibility to infection and the effects of infection on the Malpighian tubules of the mosquito. The transport physiology of the infected tubules is markedly affected in Aedes but not in Anopheles which has significance with respect to transmission potential. Broader implications may be revealed by a detailed ultrastructure study of mosquito osmoregulatory organs is being conducted. Relevant findings could indicate the need to evaluate the ecology of various mosquito species. Whether microfilariae can develop within a mosquito may be determined by the ability of microfilariae to avoid and/or inhibit the vector's immune response. Possibly, microfilaria avoids the vector reaction by being coated with host antigens during midgut penetration in appropriate vectors. The peritrophic membrane appears not to function as a barrier to migration of microfilariae out of the mosquito stomach. In a related study, it was shown that the peritrophic membrane and midgut epithelium of culicoids are not barriers to Dipetalonema and Mansonella microfilarial migration out of the midgut.

Brugia pahangi infection was established in rats and mice. Subcutaneous and intraperitoneal infection routes were successful in nude mice. The thymus dependence of *B. pahangi* was established. Infections in inbred and neonate mice show that immune rejection of adult and microfilarial stages depends on presence of T cell populations. Despite the success with mouse *B. pahangi* infection it was not possible to infect nude mice with *Wuchereria bancrofti*.

In a study by Japanese and U.S. scientists, the karyotypes of *B. pahangi* and *B. malayi* were analyzed and compared. Both species showed a diploid number of 10 chromosomes and by C-banding analysis the sex-determining mechanism of these species was of the XY–XX type. Both species were karyotypically indistinguishable; this further substantiates their close taxonomic relationship.

Field studies in the Dominican Republic, Haiti, Ecuador, and Colombia provided valuable information on prevalence and geographic limits of *W. bancrofti*, *Mansonella ozzardi*, and *Onchocerca volvulus*. In the Dominican Republic the highest prevalences of *W. bancrofti* positives (11.5%) occurred in the coastal plain. More than 75% of the microfilarial positives occurred in individuals under age 20. The distribution of Onchocerca infections, though in spotty loci, were greater than previously believed in South America.

SIGNIFICANT ACCOMPLISHMENTS

Schistosomiasis

1. Demonstration of protective immunity in primates against *Schistosoma japonicum* by exposure to homologous, x-irradiated cercariae.
2. Definition of the lipid metabolism of schistosomes, and of their dependence on an exogenous supply of cholesterol.
3. Elaboration of the conformational requirements for schistosomicidal activity of nitroheterocyclic drugs.
4. Establishment of new experimental models of *Schistosoma haematobium* infection in subhuman primates, with pathologic reactions similar to that of humans.
5. Expanded use of inbred strains of mice and utilization of hamsters, rabbits, and primates as animal models.
6. Establishment of the baboon as the best animal host for study of pathologic changes due to *S. haematobium* infection.
7. In baboons, the use of irradiated cercariae results in a reduction in the size of hepatic and intestinal egg-focused granulomas.
8. Eosinophils cause a significant antibody-dependent toxic effect on culture derived schistosomula.
9. Inbred mouse strains vary widely in their protection due either to chronic primary infection or irradiated-cercarial immunization.
10. Immunological mechanisms activated by schistosome eggs vary among parasite species.
11. Immunopathogenic studies showed the dependence on T-lymphocytes for schistosome ova granuloma formation.
12. A variety of immunoregulatory circuitries are known to then modulate this granuloma formation: these include antigen-specific and non-specific humoral regulation; antigen-induced suppressor T cells; antigen-specific adherent cell-dependent suppression, histamine-receptor bearing T suppressor cells, and anti-idiotypic T cell responses during infection.
13. Differences in biologic and host immune responsiveness to infections with *S. mansoni* and *S. japonicum* suggests possible differences in egg antigens between the species.

14. Development of schistosome egg antigen-specific T cell clones.

15. Hepatic fibrosis results from biologically active molecules elaborated by cells in the granulomas.

16. In schistosomiasis japonicum granulomatous reactivity and immunoregulation demonstrably involves humoral as well as cellular mechanisms.

17. Idiotypic and anti-idiotypic regulation are both important at given times after murine infection.

18. Anti-soluble egg antigen (SEA) MAbs can suppress granuloma formation, as can anti-anti-SEA MAbs.

19. A purified, cloned and expressed molecule identified as schistosome paramyosin (97 kD) is one of the major, but not sole, immunogens in crude cercarial preparation.

20. Cercarial glycocalyx may induce suppression or tolerance.

21. A cDNA clone encoding schistosome tropomyosin has been identified that shows homology to various species.

22. Mice exposed to *S. japonicum* and provided with a carcinogenic diet developed hepatocellular carcinoma.

25. Ha-*ras* related sequences were found in the DNA of schistosome.

26. Snail hemocytes react against specific sporocyst surface molecules.

27. The development of molluscan cell culture lines made new research opportunities possible.

28. The calcium regulatory protein, calmodulin, is important in schistosomes.

29. *Schistosoma mansoni* hypoxanthine quanine phosphoribosyltransferase (HGPRTase) cDNA clones have been identified and purified to homogeneity: The deduced amino acid sequence was 66% homologous with the sequence for mouse HGPRTase.

30. Adult *Schistosoma mansoni* contains at least five forms of glutathione (GSH) S-transferase (SmGST).

31. Two members of the SmGST-1/2/3 family are coded for by unique mRNAs.

32. The major secreted protease of schistosome cercariae is a serine protease released from acetabular glands in response to skin lipid.

33. A three dimensional model of the cercarial proteinase is being utilized to obtain specific information about the substrate binding specificity of the enzyme's active site.

Filariasis

1. A small laboratory animal model of lymphatic filariasis was established through infection of the jird.

2. Quantitative studies on pathogenesis of *Brugia* infections in cats were initiated.

3. It was shown for the first time that the human species, *Wuchereria bancrofti*, matured in macaque monkeys.

4. The *Dirofilaria* dog model and the various *Brugia* models showed that separate immunological mechanisms were operative in the suppression of microfilaremia versus protection from reinfection.

5. Cytoplasmic incompatibility in *Culex* related to the presence of a rickettsia-like organism, *Wohlbachia pipientis*.

6. Experimental patent infections of *Wuchereria bancrofti* in Taiwan macaques.

7. Increased levels of circulating immune complexes were noted in the sera of jirds infected with *B. pahangi*, but there was no correlation between immune complex levels and the numbers of circulating microfilariae.

8. The tricarboxylic acid cycle pathway is regarded of doubtful importance as an energy-yielding pathway in these parasites.

9. There is a potential hazard for extension of onchocerciasis and mansonellosis in new geographic areas due to exposure of greater numbers of persons through new road or land development projects.

10. The refractoriness of some *Aedes aegypti* strains for the development of *Dirofilaria immitis* microfilariae is a consequence of the environment within their Malpighian tubules.

11. Male mosquitoes have very few hemocytes and are almost totally "immunoincompetent."

12. Microfilariae form specific cyclooxygenase pathway derived prostaglandins, including prostaglandin E_2 and 6-keto-prostaglandin $F_{1\alpha}$.

13. Field trials of the efficacy of ivermectin for onchocerciasis have been very successful.

Malaria

1. The major erythrocyte receptor for *P. falciparum* is the family of sialoglycoproteins known as glycophorins.

2. Three proteinases of *P. falciparum* have been characterized whose activities were expressed stage-specifically in the life cycle of the erythrocytic parasite.

3. A cysteine proteinase has a critically important role in hemoglobin degradation in the food vacuole of trophozoites.

4. Highly specific peptide inhibitors of cathepsin L inhibit hemoglobin degradation within the food vacuole and block the multiplication of *P. falciparum*.

5. A marked dephosphorylation of host cell membrane proteins accompanies merozoite invasion.

6. The 110k rhoptry protein is secreted into the erythrocyte membrane during merozoite invasion and persists in the membrane for up to 24 hours.

7. The 140k–110k protein complex binds to mouse erythrocytes.

8. The plasmodial parasite selectively accumulates phospholipids (PL) in the rhoptry organelles.

9. The major intrinsic membrane proteins band 3 and glycophorin are transferred into parasitophorous vacuolar membrane (PVM) during the invasion process.

10. Production of a monoclonal antibody to knob-bearing erythrocytes infected with the FCR-3 strain of *P. falciparum*.

11. The monoclonal antibody partially blocked the binding of knob-bearing cells (FCR-3 strain) to formalin-fixed amelanotic melanoma cells.

12. The central lysine-rich domain of KP is the most conserved and immunogenic fragment.

13. A family of large (Mr>240,000) variable proteins on the surface of infected erythrocytes was identified.

14. The members of the cytoadherence protein family are of parasite origin and are not altered host proteins.

15. Intravenous injection of solubilized *P. chabaudi adami* antigen leads to the development of transferable, CD8+ antigen-specific suppressor cells.

16. Macrophages activated by lymphokines or recombinant gamma-IFN are able to kill intraerythrocytic *P. yoelii* malaria parasites.

FUTURE GOALS

Despite the considerable advances made in better understanding of diseases and their parasites, the ultimate goal of future control for malaria, filariasis, and schistosomiasis remains elusive. Evidence suggests that whereas control of one or more of diseases might succeed in some limited areas, the illnesses may spread in many other areas. Propagation of snail and arthropod vectors for these diseases occurs rapidly with programs to introduce water development projects into new geographic areas. This provides unfavorable environmental conditions which impact upon populations previously not troubled by such diseases. There will be a continued need for well-planned epidemiological studies to determine prevalence, incidence, and intensity of schistosomiasis and filariasis in new and old loci. Acquisition of such data will allow the design of rational programs.

The striking advances over the past several years in malaria research herald the realistic possibility of a vaccine useful for prevention. Use of molecular biological techniques found helpful in malaria research should find application for filariasis, schistosomiasis, and other vector-borne diseases. Panel-sponsored research, contract activities, and free interchange at annual joint meetings has promoted rapid exchange of information, thereby enhancing the chance for better guidelines for control of such diseases. There is need for the continued encouragement and support provided by a ready source of schistosome, filarial parasites, and vectors for Panel investigators. Also to be encouraged is the sponsorship of targeted projects for development of improved research methodologies, such as the cryopreservation of filarial parasites and development of molluscan cell lines. There were significant advances in development of effective drugs for filarial infections.

The role of the Parasitic Diseases Panels to successfully accomplish scientific advances in these globally important, vector-borne parasitic diseases should be significant and stimulating. Collaborative efforts of the Panels and their respective scientists from Japan and the United States should provide considerable progress in those numerous health problems caused by these parasitic diseases.

PROJECTS SUPPORTED BY THE PROGRAM

GRANTS

Investigator and Institution	*Title*
Nina M. Agabian University of California Berkeley	Molecular Cloning of Schistosome Antigens
Masamichi Aikawa Case Western Reserve University	Electron Microscopy of Plasmodium—Host Interactions
John W. Barnwell New York University	Molecular Analysis of Plasmodium Vivax Surface Antigens
Paul F. Basch Stanford University	Reproductive Biology of Schistosomes
Christopher J. Bayne Oregon State University	Schistosome-Snail Compatibility: Underlying Mechanisms
James L. Bennett Michigan State University	Drugs or Immune Sera: Action on Schistosomules
James L. Bennett Michigan State University	HMG-COA Reductase: Role in Schistosome Egg Production
Dov L. Boros Wayne State University	Immunoregulation of the Schistosome Egg Granuloma
Timothy J. Bradley University of California Irvine	Cellular Defense Mechanisms in Mosquitoes
James E. Byram Harvard University	Schistosome Vitellogenesis and Eggshell Formation
Ioav Z. Cabantchik Hebrew University of Jerusalem	Malaria Induced Pores in Human Erythrocyte Membranes
Brian A. Catto Medical College of Georgia	Praziquantel, Schistosomiasis & the Oxidation Phenotype
John P. Caulfield Brigham and Women's Hospital	*S. mansoni*: Immune Induced Surface Changes
Bruce M. Christensen University of Wisconsin Madison	Immune Response of Mosquitoes to Filarial Worms
Daniel G. Colley Vanderbilt University	Immunologic Responses to *Schistosoma mansoni*
Eain M. Cornford University of California	Transport Mechanisms in the Schistosome Integument
Catherine A. Crandall University of Florida	Brugian Filariasis in the Ferret
Richard D. Cummings University of Georgia	Glycoprotein Oligosaccharides of *Schistosoma mansoni*

Raymond T. Damian University of Georgia	Interplay of Immunity and Oviposition in Schistosomiasis
George M. Davis Academy of Natural Sciences	Analysis of Molluscan Faunas and Asian Schistosomiasis
Barbara L. Doughty Texas Agriculture and Mechanical University College Station	Immunoregulation in Human Schistosomiasis
Marc H. Dresden Baylor College of Medicine	*Schistosoma mansoni*—Cysteinyl Proteinase of Eggs
Marc H. Dresden Baylor College of Medicine	*Schistosoma mansoni*: Acidic Proteinase of Adult Worms
Mahmoud H. El Kouni Brown University	Nucleotide Metabolism in Schistosomes
Sharon K. File University of Puerto Rico Rio Piedras	Transit of Schistosome Eggs Through Venular Endothelium
Ernst A. Friedheim Rockefeller University	Synthesis and Evaluation of Macrofilaricidal Compounds
Jay R. Georgi Cornell University Ithaca	Migrations and Mortality of Schistosoma
Nigel G. Godson New York University	Molecular Biology of the Malaria Organism Plasmodium
Williard O. Granath Jr. University of Montana	Molecular Biology of Schistosome Snail Interactions
Kasturi Haldar Stanford University	Receptor Mediated Pinocytosis in Plasmodium
Bruce Hammerberg North Carolina State University Raleigh	Pathogenesis of Filarial Lymphademopathy
Donald A. Harn Jr. Harvard University	Vaccination Against *S. mansoni* With Purified Antigens
Eugene G. Hayunga U.S. Uniformed Services University of Health Sciences	Characterization of Schistosome Surface Antigens
Gene I. Higashi University of Michigan Ann Arbor	Eosinophil Monoclonal Antibodies in Schistosome Immunity
George V. Hillyer University of Puerto Rico Rio Piedras	Defined Immunity Antigens in Schistosomiasis
Shu Y. Hsu University of Iowa	Attrition of Migrating Schistosomula in Skin
Joseph W. Inselburg Dartmouth College	Isolation and Study of Mutants of *Plasmodium falciparum*
Joseph W. Inselburg Dartmouth College	*Plasmodium falciparum* Antigens for Vaccine Production
Stephanie L. James George Washington University	Macrophage Function in Immunity to *S. mansoni* Infection

Stephanie L. James George Washington University	Studies on a Nonliving Vaccine Against *S. mansoni*
Walter M. Kemp Texas Agricultural and Mechanical University College Station	Studies of the Tegumental Surface of *Schistosoma mansoni*
Araxie Kilejian Public Health Research Institution of the City of New York	Host-Parasite Interactions in Malaria
Thomas R. Klei Louisiana State University Baton Rouge	Lymphatic Lesion Pathogenesis in *Brugia*-Infected Jirds
Paul M. Knopf Brown University	Candidate Vaccine Protein Antigens of *S. Mansoni*
Norbert H. Lanners Tulane University	*In Vitro* Cultivation/Malaria Parasite *Plasmodium vivax*
James H. Leech University of California San Francisco	*Plasmodium falciparum* Erythrocyte Surface Antigens
Fred A. Lewis American Foundation for Biological Research	Immunity in a *Schistosoma mansoni* Abortive Infection
Phillip T. LoVerde State University of New York Buffalo	Anti-Idiotype Vaccine Development for Schistosomiasis
Phillip T. LoVerde State University of New York Buffalo	Toward A Molecularly-Defined Vaccine for Schistosomiasis
Phillip T. LoVerde State University of New York Buffalo	Cercaria-Schistosomule Transformation
Phillip T. LoVerde State University of New York Stony Brook	Molecular Basis for Schistosome Reproductive Development
James H. McKerrow University of California San Francisco	Characterization of Cercarial Proteinase of *S. mansoni*
Glenn R. Olds Miriam Hospital	Modulatory Network Components in *S. japonicum*
Margaret E. Perkins Rockefeller University	Biochemical Studies on Erythrocyte Invasion by Plasmodia
S. Michael Phillips University of Pennsylvania	*S. mansoni* Vitellogenesis and Granuloma Formation
S. Michael Phillips University of Pennsylvania	*In vitro S. mansoni* Fecundity and Granuloma Formation
S. Michael Phillips University of Pennsylvania	The Idiotypic Regulation of Resistance to Schistosomiasis
Thiruchandurai V. Rajan University of Connecticut Health Center	Molecular Biology of Human Filarial Parasites

Philippe A. Rossignol Oregon State University	Mosquito Probing Behavior and Malaria Epidemiology
Rupert K. Schmidt-Ullrich New England Medical Center Hospitals, Inc.	Mechanisms of *P. falciparum* Merozoite Invasion
Irwin W. Sherman University of California Riverside	Erythrocyte-Endothelial Interactions in Malaria
Andrew Spielman Harvard University	Mechanism of Filarial Penetration into Vectors
Miguel J. Stadecker Tufts University	Immunoregulation in Schistosomiasis
Abram B. Stavitsky Case Western Reserve University	Immune Regulation in Schistosomiasis japonica
Mette Strand Johns Hopkins University	Immuno-Protective and Pathogenic Schistosome Antigens
Theodore F. Taraschi Thomas Jefferson University	Membrane Trafficking in Malaria-Infected Erythrocytes
Diane Wallace Taylor Georgetown University	Rodent Malarial Antigen—PY117
Terrie E. Taylor Michigan State University	Immunotherapy in Pediatric Cerebral Malaria
James W. Tracy University of Wisconsin Madison	Biochemistry of Schistosome Glutathione S-Transferases
James W. Tracy University of Wisconsin Madison	Role of Glutathione in Drug Metabolism by Schistosomes
William Trager Rockefeller University	Extracellular Development *in vitro* of Malaria Parasites
Carmelita U. Tuazon George Washington University	Nonliving Vaccine Against *S. mansoni*
Akhil B. Vaidya Hahnemann University	Organelle Genomes of Malarial Parisites
David L. Vander Jagt University of New Mexico Albuquerque	Protein Degradation in *P. falciparum*
Jerome P. Vanderberg New York University	*Plasmodium* Sporozoite—Host Cell Interactions
Anne C. Vezza University of Alabama Birmingham	Transcriptional Analyses of the *P. falciparum* RNA Genes
Anne C. Vickery University of South Florida	Filarial Immunology in Nude Mice
Franz C. Von Lichtenberg Harvard University	Schistosomiasis and Granulo [illegible]tion

John H. Waite University of Delaware	Eggshell Precursor Proteins in *Fasciola* and *Schistosoma*
Ching C. Wang University of California San Francisco	Purine Metabolism in *Schistosoma mansoni*
Donald L. Wassom University of Wisconsin Madison	Immunoregulation in Murine Malaria
Gary J. Weil Washington University	Circulating Parasite Antigens in Human Filariasis
Gary J. Weil Jewish Hospital of St. Louis	Infective Larvae and Immunity in Filariasis
Peter F. Weller Beth Israel Hospital (Boston)	Arachidonic Acid Metabolism by Filarial Parasites
David J. Wyler Tufts University	Fibroblast Stimulation in Schistosomiasis
Mariano G. Yogore Jr. University of Chicago	Philippine Schistosomiasis japonica: Immune Responses
Timothy P. Yoshino University of Oklahoma Norman	Immunobiology of Schistosome-Mollusc Interactions

CONTRACTS

John I. Bruce University of Lowell	Supply of Schistosome-Infected Snails and Mammals
John W. McCall University of Georgia	Filariasis Repository Research Service

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Japanese Parasitic Diseases Panel

BROAD GOALS

At the inception of the Program, the Joint Panels decided to focus their efforts on two important helminthic infections, schistosomiasis and filariasis. In 1972, the Review Committee of the Program suggested revising guidelines based on the Program's progress. However, in 1975, the Joint Panels reaffirmed their desire to confine activities to these two diseases rather than to disperse efforts on multiple areas. Both Panels continued to carefully review their research activities and discuss the guidelines at each joint meeting. In 1983, these objective discussions resulted in a guideline change with the inclusion of malaria and other hemoprotozoan diseases. The current guidelines are presented at the beginning of this section.

PROGRESS IN PRIORITY AREAS

Schistosomiasis

Epidemiology

No new human or animal cases of schistosomiasis have been found in Japan since the last case in the Kofu Basin in 1977.

A new habitat of *Oncomelania nosophora* was found in 1985 in haute Obitsu River Basin in Boso Peninsula on the mainland Honshu, Japan. In this region several cases of chronic hepatitis due to schistosome infection were detected by chance. The inhabitants of the river basin were then examined for schistosomiasis by the combination of ELISA, CFT, COP, Ouchterlony and by stool examinations. No egg positive cases were present, although 70 (19.7%) of 356 examinees were positive to more than two of the immunological tests. The younger age group of inhabitants such as the students of the primary and junior high schools did not show any positive reactions to the serological tests. Although the snails were found abundantly in irrigation ditches, none of them were found to be infected. All evidence indicates that the transmission of schistosomiasis has terminated in this area.

Studies in and around Mwanchinga Village, Kwale, Kenya, where urinary schistosomiasis has been endemic, confirmed that availability of safe water supply partially reduced the frequency of water contact of villagers. Praziquantel was superior to metrifonate in terms of short-term efficacy against schistosomiasis hematobia but was the same as metrifonate in terms of long-term efficacy.

Vector Biology and Control

O. nosophora snails from the Kofu Basin, Japan, which has been treated persistently with NaPCP, Yurimin, and Phebrol since 1960, were compared with the snails from the Obitau River Basin, which has never received any form of snail control, to determine their susceptibility to the molluscicides. The results showed no evidence of resistance to molluscicides even after many years of mollusciciding.

Parasite Biology

Cercariometry was carried out in a village in Kenya. *S. hematobium* cercariae showed an apparent daily periodicity. Although the number of cercariae observed varied from site to site, the highest density was found at 1100 hours at all the study sites, with few cercariae present at 0900 hours or 1600 hours.

The pattern of the cercarial emergence of *O. nosophora* was studied outdoors in autumn and winter in Japan. Irrespective of the time of submerging the snails into water, the peak level of emergence occurred in the early part of the night, though the snails released a few cercariae soon after submersion.

Daughter sporocysts of *S.japonicum* obtained from experimentally infected *O. nosophora* were transplanted to noninfected *O.nosophora*. It was recognized that daughter sporocysts produced granddaughter sporocysts 4 weeks after transplantation. The granddaughter sporocysts produced cercariae 8 weeks after transplantation. The cercariae became mature and emerged 11 weeks after transplantation.

An attempt was made to verify the existence of DNA rearrangement in schistosomes in their complex alloiogenesis in snail and mammalian hosts. It was shown that DNA elimination and rearrangement took place extensively in *S. mansoni* in the course of development of the use of probes cloned randomly from *S. mansoni* egg DNA.

Praziquantel-induced hatching of *S. mansoni* eggs was inhibited by EGTA and Ca^{++} channel blocker. Calcium ionophore stimulated hatching. Incubation of eggs in a high concentration of $CaCl_2$ solution enhanced the motility of miracidia. These results suggested that Ca^{++} plays an important role in praziquantel-induced hatching of schistosome eggs.

Ultrastructural changes in the subtegumental cells during transformation of *S. japonicum* cercariae to schistosomula were studied after incubation in RPMI 1640 with 1% human serum albumin. After a 3-hour incubation, the outer membrane of tegument began to change to a 5 or 7 layered structure from a 3 layered unit membrane. During this drastic change, the nuclei of subtegumental cells and somatic cells altered gradually from heterochromatic to euchromatic, and membranous bodies in subtegumental cells were replaced by tightly packed membranous bodies with a decrease of the number of these cyton granules.

Host-Parasite Interactions

Southern hybridization of the DNA of *S. japonicum* adults and eggs and *S. mansoni* adults demonstrated the presence of the sequences homologous to the *env* sequences of mouse ectotropic and xenotropic retroviruses. Hybridization was also observed between the *gag* or *pol* region of the ectotropic murine leukemia virus and DNAs of *S. mansoni* eggs and *S. japonicum* adults. Furthermore, DNAs from *S. mansoni* eggs and *S. japonicum* eggs and adults showed sequences homologous to the mouse repetitive ones, intracisternal A particle, B1, B2 and mo-1 sequences. The mo-1 sequences in schistosome genomes were highly methylated in the CG sequence. The restriction fragment length (RFLP), using mo-1 as a probe, was different in DNAs of schistosome and ICR strain. When the host was changed to another strain of mice, these RFLPs in DNAs of the schistosome were somewhat converted. It was demonstrated that BLV, a mammalian retrovirus, was able to infect eggs of *S. mansoni*.

Pathology

Serum levels of several markers for liver fibrosis were measured in three groups of subjects with schistosomiasis in northeast Brazil. None of the sera were positive for antibodies to the surface antigen of HBs or circulating HBs antibodies to the surface antigen of HBs or circulating HBs antigen. There was no significant difference in the serum levels of N-terminal peptide of procollagen type III between the egg-positive subjects and either of the egg-negative Brazilian or Japanese immigrants, whereas the mean value of serum laminin was found to increase significantly in the egg-positive subjects. A significantly higher level of immunoreactive B-subunit of prolyl 4-hydroxylase was also observed in the egg-positive subjects.

Low responsiveness in egg granuloma formation in the liver of mice was induced by different immunization schedules such as 6–8 subcutaneous injections of 10,000 lyophilized eggs or an intraperitoneal injection of a large dose (60,000) of live eggs. Transfer of spleen cells from chronically infected mice into mice that had been previously injected with *S. japonicum* eggs resulted in a decrease of hepatic granuloma size. On the other hand, oral administration of 1alpha-hydroxyvitamin D3 resulted in an increase of angiotensin converting enzyme (ACE) activity in hepatic granulomas, suggesting the production of ACE by macrophages which might modify granulomatous hypersensitivity in murine schistosomiasis mansoni.

Eggs of *S. japonicum* and *S. mansoni* obtained from the livers of mice were incubated *in vitro* for 3 days and the culture fluid was added to the monolayer of BALB 3T3 cells. DNAs from transformants 3 weeks in culture were isolated and digested with EcoRI or BamHI. Southern blot analysis revealed that among the oncogenes examined, only v-Ha-*ras* insert DNA (0.46 kbp) was able to detect some additional signals in DNAs of transformants. The presence of Ha-*ras* related sequences was shown in the DNA of the schistosome. On the other hand, amplified Ha-*ras* sequence was detected in the DNA of a hepatoma caused by chronic infection with *S. japonicum*.

Immunology

Immunohistochemical analysis was made by an avidinbiotinated horseradish peroxidase complex procedure using goat antisera against various viral products or the whole virions of mouse retroviruses and adults of *S. japonicum* and *S. mansoni* obtained from mice. The specific reaction with the parasites was observed by using the antiserum against gp70 (glycoprotein produced by the *elv* gene) of mouse xenotropic retrovirus, BALB virus 2(Bv2). This reaction was completely inhibited by preabsorption of the antiserum with virions of retrovirus. The antigen was located in the subtegumental layer of the adult fluke and the vitelline gland of the adult female of *S. japonimum* and *S. mansoni*.

A mouse hybridoma that produces an IgE antibody specific for *S. japonicum* has been established. The antibody recognized a 97 KDa antigen expressed on the surface of a mechanically transformed schistosomula and a partial but significant protection was induced by passive transfer of antibody when antibody was administered in the early stage of challenge infection. Eosinophil-mediated damage to schistosomula was observed *in vitro* in the presence of the monoclonal IgE antibody.

Low responsiveness of C57BL/6 mice in protective immunity against *S. japonicum* infection was demonstrated by using a vaccine model. In addition, peritoneal macrophages of C57BL/6 mice showed only a marginal killing activity against mechanically transformed schistosomula in the presence of a monoclonal IgE antibody to *S. japonicum* (SJ18e.1), in marked contrast to macrophages of DBA/2, a responder strain.

Immunopathological properties of human T lymphocyte clones (CD3+, CD4+, CD8−) specific for *S. japonicum* soluble egg antigen (SEA) were examined by using three fractions of SEA with different molecular weight. All clones were reactive to Fraction II(mw 7000-17000), suggesting the existence of epitope specific regulatory mechanisms in SEA-driven T lymphocyte response in man. Furthermore, at least two distinct T cell epitopes were demonstrated in Fraction II. Out of 8 clones tested, three were involved in delayed hypersensitivity to schistosome eggs *in vitro*. These results suggest that Fraction II is responsible for induction of immunopathological T lymphocyte response in human hosts.

Patients with post-schistosomal liver cirrhosis (PSLC) showed a high response to *S. japonicum* antigen, in terms of T cell proliferative response, *in vitro*. The low response to *S. japonicum* antigen, statistically associated with HLA-Dw12-DR2-DQw1, was mediated by CD8+ suppressor T cells specific for *S. japonicum* antigen. Thus, the HLA-Dw12-DR2-DQw1 haplotype is a nonresponder haplotype to *S. japonicum* antigen that controls the resistance to PSLC, through antigen-specific suppressor T cells.

The ability of T cell line YY and autologous APC as well as HLA-DR2 shared allogeneic APC to respond to *S. japonicum* antigen was markedly blocked by anti-HLA-DR monoclonal antibody(mAb) and was not affected by anti-HLA-DQw1 or by anti-HLA-class I mAbs. These findings confirmed that the HLA-DR2 molecule plays a role as a restriction molecule in the antigen presentation by APC to CD4+ T Cells, even through the DR2 originated in a nonresponder haplotype. A blocking study using various anti-HLA mAbs revealed that the nonresponse of whole mononuclear cells (WMN) of nonresponders was restored by the addition of anti-HLA-DQw1 mAbs. The restored response was at the same level as that obtained when CD8(+) suppressor T cells were removed from the culture. Because the anti DQmAb did not directly enhance the response of the T cell line on *S. japonicum* antigen or of WMN to other nominal antigens, it is most probable that the anti DQql mAb blocked the *S. japonicum* antigen-specific immune suppression. In the nonresponder haplotype, the DR2 molecule could present antigen to CD4(+) T cells to respond to *S. japonicum* antigen but this immune response was suppressed due to the presence of suppressor T cells controlled by the DQw1 molecule in the same nonresponder haplotype. In other words, HLA-DQ is epistatic to HLA-DR in regulation of the immune response to *S. japonicum* antigen.

One laboratory established HLA class II transgenic mice. A transgenic mouse to which HLA-DQ alpha and beta gene were introduced expressed HLA-DQw1 molecule in thymus, spleen, lung alveolar macrophage, and dermal cells. The B cells of the DQ transgenic mouse stimulated a mixed lymphocyte reaction (MLR) of the C57BL/6 mouse. The immune responsiveness to schistosomal antigen was measured by the *in vitro* T lymphoproliferative response of lymph node cells from mice immunized subcutaneously by 25ug of crude worm antigen.

Biochemistry

Excretory end-products of adult *S. japonicum*, fed D-($13C_6$) glucose *in vitro* under aerobic and anaerobic conditions, were studied using ^1H- and ^{13}C-nuclear magnetic resonance (NMR) spectroscopy. The presence of (13C3) and 2.3(13C2) lactate, and 1,2,2,'(13C3) and 2,2'(13C2) succinate as endproducts suggests that a partial reversed TCA cycle is active in the adult worms under anaerobic conditions. With eggs of *S. japonicum*, the endproducts under aerobic conditions were found to be lactate and alanine. The young sporocysts produced pyruvate, acetate, and succinate besides lactate and alanine under both aerobic and anaerobic conditions.

Chemotherapy

Efficacy of levo- and dextro-paraziquantel (PZQ), optic isomers of PZQ, on worm reduction and oviposition was compared with PZQ in mice infected with *S. japonicum* or *S. mansoni*. It was found that levo-PZQ was effective against *S. japonicum* whereas the isomer as well as PZQ was less effective against *S. mansoni* but not so against *S. japonicum*. Ultrastructural observations demonstrated degeneration of the genital organs of *S. japonicum* treated with levo-PZQ in addition to previously known damage of the tegument caused by PZQ.

Filariasis

Epidemiology

In San Vicente Pacaya, Guatemala, fly-bite frequency on the skin and the intensity of transmission of filariasis was statistically analyzed. The study revealed that the number of fly-bites in the upper body roughly reflected the incidence rate by age and sex.

In the endemic areas of onchocerciasis in Ecuador, a comparative study was conducted on the transmission potential of three species of blackfly. An experimental infection study revealed that *Similium exiguum* has high susceptibility to *Onchocerca volvulus*, while the susceptibility of *S. quadrivittatum* was very low. By using human-bait method, it was clarified that in the upper area of Rio Cayapas, *S. exiguum* was the most dominant species, while in the middle area of this river and Rio Santiago, *S. quadrivittatum* was the most dominant species. Only a few *S. pseudoantillarum* were collected in any areas surveyed. *S. exiguum* harbored the first stage larvae of *O. volvulus*, thus indicating the importance of this species in the transmission.

Vector Biology and Control

Success was obtained in inducing *S. ochraceum* to lay eggs in the laboratory, the oviposition rate and hatching rate of eggs were 55% and 72%, respectively. The lifespan of microfilariae released *in vitro* by *Dipetalonema viteae* was studied. The presence of serum in culture medium facilitated the subsequent development of larvae in the tick, *Ornithodorus tartakovski*.

The wing-sound trapping for male mosquitoes was effective in control of mosquitoes. For the species of the genus *Mansonia* the sound of 330–350 Hz together with an animal in a cage and dry ice was the most effective, while for *Aedes albopictus* the sound of 480 Hz with the animal caged and dry ice was most efficient. The combination with other strategies may hold promise for the future.

A new approach to human blood index of mosquitos was made by using the DNA hybridization technique and identification of mosquitos that have fed on men and cows is now possible.

Host-Parasite Interactions

In cotton rats infected quantitatively with 20 infective larvae of *Litomosoides carinii* the course of infection was followed up to 9 months after inoculation. Microfilarial (mf) density of infected animals was periodically measured and the fecundity of adults extirpated surgically was examined under culture conditions *in vitro*. These results were compared in order to estimate mf-killing activity of the host animal. It was disclosed that mf produced were pooled in the host animal without being damaged up to 3 months after inoculation. The mf density reached its peak 3–5 months after infection and it was gradually decreased by the killing mechanism. There are two modes in the reduction phase, one is rapid and the other is slow, though no difference exists in the worm burden in the hosts. In rats with higher mf density the OD value of ELISA was somewhat lower than those with lower mf density, while no suppressive effect of sera on mf-production was found in the former animals.

Immunology

Several monoclonal antibodies were developed against different species and different stages of filarid worms. In a laboratory 8 monoclonal antibodies against mf of *Brugia malayi* (belonging to IgG1, IgG2b, IgG3) were established and they were indicated effective to distinguish circulating mf in the mice which have been experimentally transfused with 200,00 mf. In another laboratory localization of IgE-inducing allergen and IgG-inducing antigen was examined in sectioned *Dirofilaria immitis* by the indirect immunofluorescence method. Allergen was found in the body cavity fluid and excretory canal, while the IgG-inducing antigen was in the musculature layer and the inner layer of cuticle.

In an old bancroftian filariasis focus in Goto Island, Nagasaki, it was demonstrated that the antibody titer against filarial antigen was significantly higher among adult T cell leukemia (ATL) patients than among ATL virus uninfected persons, both males and females. The mechanism involved in the transformation of T cell must be investigated.

A total of 17 monoclonal antibodies against *O. volvulus* was used to search for the antigenic similarity of *O. volvulus* to other 13 species of parasitic helminths by immunofluorescence. The cluster analysis of the results obtained indicated that *Ascaris lumbricoides suum* was the closest species to *O. volvulus* in antigenic composition, and a possibility that some proxy antigens would be attained from *A. lumbricoides suum* in lieu of *O. volvulus*.

Eosinophil response to filarial infection was studied. In *Brugia pahangi*-jird and *Litomosoides carinii*-cotton rat models, eosinophil response is suppressed when animals have become patent infected. In *B. pahangi*-BALB/c or C57BL/6 model, eosinophil and macrophage play an important role in the protective immunity.

A high protective immunity (100%) was induced in BALB/c mice against *B. malayi* by immunization with 500.00 mr emulsified in FCA. Likewise, a high protection (95%) was also induced in C3H/HeN mice, while only 19% protection was formed in C3H/HeJ. The degree of protection in such C3H/He substrains did not correspond to their response to LPS.

Microfilarial antigens were affinity purified using 4 kinds of monoclonal antibodies (mAbs) against *B. malayi*. Two of them induced relatively high protection (71% and 56%) in BALB/c mice by immunization. The mAbs were screened to detect circulating antigens in blood using avidinbiotin ELISA. A mAb, detectable to circulating *B. malayi* antigens, was not specific to *B. malayi* but cross-reactive to *Litomosoides carinii*, *B. pahangi*, *Dirofilaria immitis*, or *Paragonimus westermani*. The molecular weight of the antigen detected by the mAb was 40 KDa.

Chemotherapy

Milbemycin-D was given to cotton rats intrathoraciacally as a single dose of 1 mg/kg, and the infective larvae of *Litomosoides carinii* were inoculated into the animals. This study was designed to examine its activity particularly against the larvae. The drug exhibited a remarkable inhibition of the larval growth being 5 times as strong in antifilarial activity as Flubendazole. Microfilaricidal action of Milbemycin-D was examined for *Dipetalonema viteae* in hamsters by administering subcutaneously at a dose of 100 mg/kg for 5 consecutive days. The treatment resulted in 75% reduction in worm recovery.

An *in vitro* study on the mode of action of diethylcarbamazine (DEC) revealed an antibody-independent elimination of microfilariae from the blood of mice infected with *B. pahangi*. The infective *B. pahangi* larvae exposed to DEC *in vitro* seldom developed or survived in jirds.

It was shown that DEC inhibited polymerization of tubulin extracted from fresh porcine brains and caused depolymerization of tubulin. An electron microscopic observation revealed unraveling of the end of assembled microtubules in DEC solution.

Malaria and Other Hemoprotozoan Diseases

Epidemiology

Of 1,054 man-biting sandflies from the Department of Canar, Ecuador examined for leishmanias, 2 species, *Lutzomyia trapidoi* and *L. hartmanni*, were found infected. The rates of infection were 7.7% in the former and 3.9% in the latter species. Six man-biting species of sandflies were collected in a human bait survey at 7 study areas in 5 Departments.

Indirect fluorescent antibody test(IFAT) was found efficient and applicable to immunodiagnosis for Japanese cases of malaria. IFAT using *P. falciparum* and *P. vivax* antigens was conducted on 53 Japanese blood positive malaria cases. Coincidence of species diagnosis between blood examination and IFAT was almost complete, showing a coincident rate of 23/24 for *P. falciparum* and 26/26 for *P. vivax*. Fifty-eight Japanese volunteer students who had never been abroad were tested but none were positive in IFAT.

Leishmania spp. collected in Ecuador were examined by isoenzyme method and immunological technique using monoclonal antibodies and they were identified as follows: *Leishmania* isolated from *Potos flavua*, *Sciurus vulgaris*, and *Tamandua tetradactyla* was *L. amazonensis*, that from 3 patients was *L. panamensis*, and that from *Sciurus granatensis* and 2 patients was a *L. major*-like parasite.

Parasite Biology

P. falciparum grows within human red cells, in which low levels of Na$^+$ and high levels of K$^+$ are maintained by the membrane Na$^+$, K$^+$-ATPase. On the other hand it is revealed that mouse erythrocytes infected with *P. yoelii* have significantly reduced K$^+$ contents with concomitant increase in Na$^+$ contents. Whether or not high levels of K$^+$ and low levels of Na$^+$ in erythrocyte cytoplasm are necessary for the growth of *P. falciparum* were then examined. When human red cells were incubated with ouabain, an inhibitor of Na$^+$, K$^+$-ATPase, in order to increase Na$^+$ contents, the treated red cells indeed increased Na$^+$ contents and reduced K$^+$ contents considerably, and the Na$^+$/K$^+$ ratio reversed 4 to 6 days after incubation. However, the presence of ouabain in culture did not inhibit the growth of *P. falciparum* as checked by daily examination for parasitemia. These results may indicate that the growth of *P. falciparum* does not depend on high levels of K$^+$ and low levels of Na$^+$ found in the normal erythrocyte cytoplasm.

In order to obtain fundamental knowledge concerning membrane transport of cations in malaria-infected erythrocytes, cloning of genes for cation-transporting ATPase of *P. yoelii* was attempted. A genomic DNA library of the parasite was constructed using ZAP. Several clones were gotten by screening the library with synthetic 21-mer oligonucleotide, the sequence of which was derived from genes for ATPases of other organisms. Partial sequencing revealed that one clone had a high homology with rabbit sarcoplasmic Ca2^{++} ATPase.

A trial to induce gametocytogenesis in cultured *P. falciparum* was carried out and it was found that the culture supernatant of anti-*P. falciparum* antibody-producing hybridoma cells was capable of inducing the gametocytogenesis. It was also proved that human serum in *P. falciparum* culture could be replaced by horse serum. The gametocytogenesis-inducible substance was proved to be heat-stable, insoluble in organic solvents, water soluble, and of smaller molecule than 1,000.

Progress has been made in studies on the nature of a gene for polymorphic surface antigen of *P. falciparum* merozoite (p190). It was revealed that variable sequences were not sequestered in the genome as silent copies akin to those of the variant specific surface antigen gene of *Trypanosoma*, that the p190 gene existed in a single copy of the haploid genome of the parasite, and that the intragenic recombination of alleles created polymorphism of the surface protein.

Attempts were made to pinpoint the target(s) for acivicin in pyrimidine and purine biosynthesis in the trypanosomatids and to assess the mechanism of growth inhibition of organisms by this compound. It was shown that in pyrimidine and purine biosynthetic pathways in trypanosomatids, glutamine-dependent key enzymes, GMP synthetase and CTP synthetase, were actually operative, and that acivicin primarily targeted these two enzymes, leading to the inhibition of CTP, GTP, and dGTP syntheses and consequently to the inhibition of RNA and DNA syntheses. Acivicin appeared to be cytotoxic to *Leishmania mexicana in vivo*.

Chemotherapy

In studies designed to search a possible mechanism for antimalarial action of chloroquine and related drugs, it was demonstrated that the ferr-proto-porphyrin IX(FPIX)-chloroquine complex remarkably promoted peroxidative cleavage of unsaturated phospholipids in liposomes, compared with FPIX alone. Quinine, mepacrine, or mefloquine also made a coordination complex with FPIX. Each complex then induced the membrane lipid peroxidation more powerfully than FPIX alone. On the other hand, primaquine which is not so effective in killing the parasites in endoerythrocytic stages, neither coordinated with FPIX nor promoted the lipid peroxidation.

SIGNIFICANT ACCOMPLISHMENTS

Since the inception of the Japan-U.S. Cooperative Medical Science Program in 1965, the focus of the Joint Panels on Parasitic Diseases has been restricted to schistosomiasis and filariasis. In 1984, both the Japanese and U.S. Panels revised the guidelines that expanded their research to malaria and other vector-borne hemoprotozoan infections.

Schistosomiasis

Epidemiological surveys for schistosomiasis were conducted intensively in Japan and Asian, African, and South American countries.

Control of schistosomiasis in Japan was successfully achieved during the first 10 years of the Japan-U.S. Program by means of well-planned vector snail control; i.e., through the lining of all irrigation ditches in ricefields with concrete and the application of molluscicides. In addition, overall rise in economic and social standards cannot be overlooked. As a result, no new human cases have been found in this country since the last case in the Kofu Basin in 1977.

A new habitat of *Oncomelania nosophora* was found in 1985 in the Obitsu River Basin in Boso Peninsula on the mainland Honshu, Japan. Although the snails were found abundantly in irrigation ditches, none of them were found to be infected. All evidence may indicate that the transmission of schistosomiasis, if it ever existed, has already been terminated in this area.

In the Philippines, seven new colonies of the vector snail *O. quadrasi* were discovered on Bohol Island in addition to six locations from which the snails were previously reported. Mass chemotherapy with praziquantel on Bohol Island remarkably reduced transmission as measured by incidence. The uneven distribution of the snail, *O. quadrasi*, was thoroughly studied and the snail survey data were successfully normalized by a simple transformation formula, $\log(x+0.01)$. Studies on molluscicides have been carried out with phebrol, a synthetic compound, and with the seed of *Jatropha curcas*, a plant molluscicide.

In vitro culture media were improved for *S. japonicum* adults, eggs and miracidia, and for the midgut tissues of *O. nosophora*. The killing effect of monoclonal antibodies of IgG1, IgG2a, and IgE, neutrophils, eosinophils, and complement on schistosomula *in vitro* was studied. Daughter sporocysts of *S. mansoni* and *S. japonicum* have been successively transplanted into *B. glabrata* and *O. nosophora*, respectively.

An extraordinary molecular biological discovery was a striking DNA sequence homology between the envelope glycoprotein sequence of murine retrovirus proviral DNA (*env*) and genomic DNA of *S. japonicum* and *S. mansoni*, as well as homology between *gag* and the eggs of *S. mansoni* and a portion of the *pol* sequence and adult forms of *S. japonicum*. Recombinant DNA fragments derived from the *S. mansoni* eggs did not hybridize with DNAs of male or female adult worms of *S. mansoni* suggesting rearrangements and deletions of DNA sequences occurs during development of *S. mansoni*.

A mouse hybridoma that produces an IgE antibody specific to *S. japonicum* has been established. The antibody recognized a 97 KDa antigen expressed on the surface of mechanically transformed schistosomula and a partial but significant protection was induced by passive transfer to the antibody when administered in the early stage of challenge infection.

In populations residing in an endemic area at the Kofu Basin, Japan, low responsiveness to schistosome antigen in T cell proliferation response *in vitro* was found to be associated with HLA-Dw12-DR2-Dqw1 haplotype. Low responsiveness was controlled by antigen-specific CD8+ suppressor T cells. Further studies using monclonal antibodies to HLA-class II antigens and long-term cultured T cell lines revealed that the HLA-DQw1 molecule of the nonresponder haplotype is a product of the immune suppression (Is) gene and that the molecule plays a major role in the antigen-specific suppression to *S. japonicum* antigen by overcoming the function of the DR2 molecule, a product of immune response gene which acts as a restriction molecule in interactions between T cells and monocytes to respond to the antigen. A significant decrease in the frequency of this particular HLA haplotype as well as a significant increase in that of HLA-Dw19-DRw13-DQw1 haplotype was observed in patients with post-schistosomal liver cirrhosis. These patients showed a high response to *S. japonicum* antigen, suggesting that the Is gene controls the resistance to induction of liver cirrhosis due to *S. japonicum* infection.

Blood added to filter paper and then stored in a desiccator, even at room temperature in the Tropics, was a satisfactory source of antibody. Several serological methods for diagnosing schistosomiasis have been developed that are highly reliable. Hemoglobin specific protease exhibited species specificity in RAST.

Clinical observations on cerebral and hepatosplenomegalic types of schistosomiasis were done in Japan and in the Philippines. Characteristic electroencephalograms were found in both areas.

The etiology of cerebral schistosomiasis was found to be due to the egg embolism and/or vascular changes in the brain. The action of praziquantel on forced shedding of miracidium from the egg was clarified. *S. japonicum* was highly susceptible to levo-praziquantel, an optic isomer of praziquantel, but *S. mansoni* was not.

In the study on the *de novo* pathway of pyrimidine synthesis in *S. mansoni*, carbamoylphosphate synthetase was found to play a key role in the initial steps. A unique cyanide insensitive pathway was found in a mitochondria fraction of *B. galbrata*.

Filariasis

The control of human filariasis in Japan was completed after a 1962–69 campaign using selective mass treatment with diethylcarbamazine (DEC). Improvement of the filtration technique for microfilaria (mf) detection made the survey efficient and satisfactory. The methods of data analysis for mf density and periodicity in a locality were devised for characterizing the local epidemiological features. In an old bancroftian filariasis focus in Goto Island, Kyushu, it was demonstrated that the antibody titer against filarial antigen was significantly higher among adult T cell leukemia (ATL) patients than among ATL virus uninfected persons of both sexes.

Since 1973, epidemiological studies were performed on onchocerciasis in Guatemala. The prevalence of mf in the skin and eyes was 32.2% and 6.2%, respectively. The annual mf incidence observed in the skin was 6.4% and 3.5% in males and females, respectively. Serologic methods for diagnosis were advanced fairly well using various antigens. Antigens derived from adult *D. immitis*, specifically IgG and IgE, were separated and their chemical characteristics were analyzed.

In the endemic areas of onchocerciasis in Ecuador, it was observed that in the upper area of Rio Cayapas, *S. exiguum* was the most dominant species, while in the middle area of this river and Rio Santiago, *S. quadrivittatum* was the most dominant species. Only a few *S.pseudoantillarum* were collected in any survey area.

A new approach to human blood indexing of mosquitoes was made by using DNA hybridization technique, and identification of mosquitoes fed on man and cows is now possible.

IgE-inducing allergen was found in the body cavity fluid and excretory canal in sectioned *D. immitis*, while the IgG-inducing antigen was in the musculature layer and the inner layer of cuticle. In *B. pahangi*-BALB/c or C57BL/6 model, eosinophils and macrophages played an important role in the protective immunity. A high protective immunity was induced in BALB/c and C3H/HeN mice against *B. malayi* in immunization with 500,000 mf emulsified in FCA. Only 19% protection was formed in C3H/HeJ.

Of two types of b-cytochrome functioning in the electron transport system of *D. immitis*, one was oxidizable by fumarate. This indicated the presence of an alternate electron transport system employing fumarate as the hydrogen acceptor.

Several animal models of filariasis were established which strengthened pathophysiological and therapeutic studies. The results of studies on the mechanism of action of DEC indicated that immunological conditioning together with macrophage activity was required for the drug to be active. DEC inhibited polymerization of tubulin extracted from fresh porcine brains and caused depolymerization of tubulin. An ultrastructural observation revealed unraveling of the end of assembled microtubules in DEC solution. Milbemycin-D exhibited a remarkable inhibition of the larval growth of *L. carinii* being 5 times as strong in antifilarial activity as Flubendazole.

Malaria and other hemoprotozoan diseases

P. falciparum grows within human red cells, in which low levels of Na$^+$ and high levels of K$^+$ are maintained by the membrane Na$^+$, K$^+$-ATPase. Its growth did not depend on high levels of K$^+$ and low levels of Na$^+$ found in the normal erythrocyte cytoplasm.

Progress has been made in studies on genetic dimorphism in a surface antigen gene of *P. falciparum* merozoite (termed p190 or gp195), which is a candidate for a malaria vaccine. The complete nucleotide sequence of the MAD20 p190 gene was determined using cloned genomic DNA. The gene was AT-rich (75%) and had a single open reading frame coding for 1,701 amino acids. Its predicted size (194 kDa) was greater than p190 from the K1 and Wellcome strains (188 kDa) previously reported. The protein had a putative signal peptide at the N-terminus, followed by a group of tandem repeats of tripeptide, and a cluster of hydrophobic amino acids at the C-terminus. Comparisons of the MAD20 p190 gene with that from the K1, Wellcome, and FUP strains revealed that the gene consisted of several variable blocks of sequences flanked by conserved or semiconserved blocks. Southern blot and hybridization studies using variable and constant fragments of the p190 gene demonstrated that each strain of *P. falciparum* had only a copy of the p190 gene in the genome and that variable sequences were not sequestered as silent copies akin to those of the variant specific surface antigen gene of *Trypanosoma*. The p190 gene existed in an allelic form exhibiting dimorphism.

In pyrimidine and purine biosynthetic pathways in trypanosomatids, glutamine-dependent key enzymes, GMP synthetase and CTP synthetase, were actually operative. Acidvicin primarily targeted these two enzymes, leading to the inhibition of CTP, GTP, and dGTP syntheses and consequently to the inhibition of RNA and DNA syntheses.

FUTURE GOALS

Panel-sponsored activities were expanded in 1984 to include research on malaria and other vector-borne protozoan infections. At that time newer technologies, particularly the use of monoclonal antibodies and recombinant DNA technologies had already been introduced into the study of malaria. It was thus anticipated that these new molecular and genetic tools and approaches found helpful in malaria research should find application for the vector-borne helminthiases, schistosomiasis, and filariasis.

As has been evident in the past 5 years, the highly interactive activities by scientists of both Panels on Parasitic Diseases have been fruitful in integrating the most modern molecular genetic and immunological approaches to attacking these major tropical diseases. Efforts will continue to increase understanding of the vector-borne helminthic and protozoan infections, thereby allowing development of effective modes of prevention or treatment of these major worldwide threats to human health. Strategies include vector control, interruption of the parasite lifecycle, and reduction of the amount and effects of infection in man by vaccination, chemoprophylaxis, or other modalities.

PROJECTS SUPPORTED BY THE PROGRAM

Investigator and Institution	*Title*
Dr. Yoshiki Aoki Nagasaki University	Epidemiology of filariasis and schistosomiasis
Dr. Koichiro Fujita Tokyo Medical and Dental University	Immunology of filariasis
Dr. Shigeo Hayashi National Institute of Health	Epidemiology and immunololgy of filariasis and schistosomiasis
Dr. Akira Ishii Okayama University	Immunology of schistosomiasis
Dr. Tozo Kanda St. Mariana University	Epidemiology of filariasis and biology of the vector of filariae
Dr. Yoshimasa Kaneda Tokai University	Biochemistry of hemoprotozoal diseases
Dr. Somei Kojima Chiba University	Immunology of schistosomiasis
Dr. Tsutomu Koyama National Institute of Health	Biology of hemoprotozoa
Dr. Toshio Nakabayashi Fujita-Gakuen Health University	Biology of malaria
Dr. Tomoo Oshima Yokohama City University	Pathology of schistosomiasis
Dr. Hiroshi Oya Juntendo University	Biochemistry of schistosomiasis
Dr. Takehiko Sasazuki Kyusyu University	Immunogenetics of schistosomiasis
Dr. Atsuo Sato Kagoshima University	Immunodiagnosis and epidemiology of filariasis and schistosomiasis
Dr. Fujiro Sendo Yamagata University	Immunology of schistosomiasis
Dr. Toshiro Shibuya Teikyo University	Immunology and chemotherapy of filariasis
Dr. Mamoru Suzuki Gunma University	Studies on malaria vaccine
Dr. Isao Tada Kumamoto University	Immunodiagnosis and epidemiology of filariasis and schistosomiasis
Dr. Suehisa Takada Osaka City University	Biology of hemoprotozoa and filariae
Dr. Tsutomu Takeuchi Keio University	Pathophysiology of hemoprotozoal diseases
Dr. Kazuyiki Tanabe Osaka Institute of Technology	Molecular biology of malaria

Dr. Hiroshi Tanaka University of Tokyo	Epidemiology, immunology, and chemotherapy of schistosomiasis and filariasis
Dr. Moriyasu Tsuji Hiroshima University	Immunodiagnosis of schistosomiasis, laboratory breeding of *Oncomelania* snails
Dr. Hiroshi Tsutsumi Kurume University	Pathology and immunology of schistosomiasis
Dr. Yoshito Wada Nagasaki University	Biology and control of vectors of filariae
Dr. Hisashi Yamamoto Dokkyo University	Biology of vectors of filariae
Dr. Toshio Yanagisawa Kitasato University	Biochemistry of schistosomiasis and filariasis
Dr. Kazuo Yasuraoka University of Tsukuba	Biology and control of schistosomiasis

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Joint Scientific Conferences

PARASITIC DISEASES PANELS

Date: August 12–14, 1985

Place: East Lansing, Michigan

Summary: The 20th Joint Conference of the Panels on Parasitic Diseases was held at the Kellogg Center of Michigan State University and was attended by approximately 60 participants, 6 of whom were from Japan. Thirty-three scientific papers were presented on aspects of the immunology, pharmacology, biochemistry, and molecular biology of schistosomiasis, filariasis, and malaria. Definition of protective immune responses and mechanisms of antiparasitic drug action were two major topics of discussion. A highlight of the meeting was a field trip to the Kellogg Biological Field Station.

Date: September 2–5, 1986

Place: Chiba, Japan

Summary: More than 80 participants attended the 21st Joint Conference of the Panels on Parasitic Diseases, including 11 from the United States. Eighteen presentations described research on filariasis, schistosomiasis, vector biology, and malaria, with emphasis on aspects of immunopathology, immunodiagnosis, biochemistry, and molecular biology. A minisymposium was included to discuss recent advances in the potential use of monoclonal antibodies in parasitic disease research. These presentations described the use of monoclonal antibodies to define potential vaccine immunogens and protective immune mechanisms against schistosomes and filaria. The final day of the meeting included an ecological tour, in which the group was shown areas where transmission of the human lung fluke, *Paragonimus*, had recently been reported and where the snail vectors for schistosomiasis were present in land irrigated for rice farming.

Date: August 19–21, 1987

Place: Williamsburg, Virginia

Summary: The 22nd Joint Conference of the Panels on Parasitic Diseases was held in Colonial Williamsburg, with 25 participants including 7 from Japan. Twenty-six reports were presented, incorporating research on vaccine production, chemotherapy and diagnosis in schistosomiasis, filariasis, and malaria. A minisymposium entitled "Targets and tactics of drug design" featured general presentations on strategies and basic principles of antiparasitic chemotherapy, as well as specific discussions on enzymatic and physiologic targets for the rational design of drugs against nematode and trematode parasites.

Date: July 17–20, 1988

Place: Tokyo, Japan

Summary: Approximately 100 participants, 12 of whom were from the United States, attended the 23d Joint Conference on Parasitic Diseases held at the Tokyo Medical and Dental University. A total of 44 papers on schistosomiasis, filariasis, and malaria research were presented. These included descriptions of candidate vaccine immunogens for *Brugia malayi*, *Schistosoma mansoni*, and *Plasmodium falciparum*. A minisymposium on "Application of techniques in molecular biology to parasitology research" covered broad topics related to genetic control of protective response to parasites in the vertebrate host and the vector, isolation and characterization of recombinant parasite antigens, and genetic rearrangements occurring within different parasite lifecycle stages.

Date: July 31–August 2

Place: Madison, Wisconsin

Summary: The 24th Joint Conference of the Panels on Parasitic Diseases was held at the University of Wisconsin in Madison. More than 34 participants attended, including 10 from Japan, and 36 papers were presented.

In the area of malaria research, antigens involved in transmission blocking, infectivity for the human host, cytoadherence, and diagnosis were described. The role of T lymphocytes in protective immunity against malaria was also discussed. Several reports detailed mechanisms of drug action and drug resistance in schistosomiasis, filariasis, and malaria. Interactions affecting parasite development in the invertebrate host were described in schistosomiasis and filariasis. A series of molecular studies aimed at understanding mechanisms of gene expression in malaria and filaria, and at developing diagnostic probes was reported. The role of proteases in the infectivity of schistosomes and nematode parasites was also discussed.

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Dr. Ichiro Azuma, Chairman (1988– , Member 1981–)
Director
Institute of Immunological Science
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Profiles

U.S. TUBERCULOSIS PANEL

Dr. Frank M. Collins

Chairman (1986–Present)

Dr. Collins received his Ph.D. degree from the University of Adelaide, Australia in immunochemistry in 1961 and D.Sc. degree in microbiology from the same university in 1976. He is currently a member of the Trudeau Institute, Inc. He has published extensively on the immune response to persistent mycobacterial infections, the immunology of tuberculosis, the kinetics of the delayed-type hypersensitivity response in tuberculosis, etc. Dr. Collins has chaired the U.S. Tuberculosis Panel since 1986.

Dr. Mayer B. Goren

Chairman

Dr. Goren was a member of the U.S. Tuberculosis Panel 1977–1980 and Chairman, 1980–1986. Dr. Goren was born in Tomaszow, Poland and emigrated to Dallas, Texas in 1927. He studied at Baylor University, Rice University (B.A. and M.A. in chemistry, 1942, 1943), and Harvard University (Ph.D., organic chemistry, 1950). After Harvard he was Assistant Professor of Chemistry at Northeastern University, Boston, Mass., 1949–1951. He was a chemist for Kerr-McGee Oil Industries, Oklahoma City, Oklahoma from 1952 to 1963 when he moved to National Jewish Hospital in Denver, Colorado, as a research scientist in the Department of Research and Laboratories with Dr. Gardner Middlebrook. Research there involved immunological studies with *Cryptococcus neoformans* and the surface chemistry of mycobacteria—especially of lipids putatively implicated in virulence or in attenuation; structural chemistry and synthesis, utilization of mycobacterial lipids in tumor immunotherapy, and macrophage function especially as influenced by mycobacterial products. He currently is the Margaret Regan Investigator in Chemical Pathology at National Jewish Center, and Professor of Microbiology Immunology at the University of Colorado Health Sciences Center.

Dr. William R. Barclay

Chairman (1969–1974)

Dr. Barclay received his M.D. from the Univesity of Alberta, Canada, in January 1946 and joined the Canadian Indian Health Services. He served there for 3 years in a large tuberculosis hospital. In 1951, he joined the medical faculty of the University of Chicago as an instructor. He remained on that faculty until 1970 when he became director of the scientific activities for the American Medical Association. At the Univesity of Chicago he became a full professor and headed the Divisions of Chest Medicine and Infectious Diseases. In 1956, he spent a sabbatical leave at the Dunn School of Pathology in Oxford and in 1966, he spent a sabbatical year at the Rocky Mountain Laboratory of the National Institutes of Health in Hamilton, Montana, working with Dr. Ribi. He served on the editorial boards of the American Review of Respiratory Diseases and the Jounal of Infectious Diseases. He was appointed to the Tuberculosis Panel at its inception and attended the first organizational meeting in Hawaii. He followed Dr. Middlebrook as Chairman of the Panel and served for 5 years (1969–1974).

JAPANESE TUBERCULOSIS PANEL

Dr. Ichiro Azuma

Chairman (1988–)

Dr. Azuma began his research on tubercle bacilli with Professor Yamamura in the Department of Medical Chemistry, Faculty of Science, Kyushu University (1960–1962), as a postgraduate student, and continued his work with Professor Yamamura in the Osaka University Medical School (1962–1978). He has been at the Institute of Immunological Science, Hokkaido University since 1979. Dr. Azuma has made outstanding contributions to the biochemical properties and immunological activities of cord factor (trehalose-mycolate), polysaccharide antigens, and cell-wall skeletons of tubercle bacilli and related bacteria. When he was a postgraduate student in Kyushu University, he identified the linkage unit (arabinose-mycolate) of mycolic acid which was the specific fatty acid in the cell wall of mycobacteria and related bacteria. Dr. Azuma, with Dr. Yamamura, proved that the adjuvant activity of Freund's complete adjuvant was in the cell-wall skeleton (CWS) of mycobacteria, and together they developed the modality of cancer immunotherapy using BCG-CWS and *Nocardia rubra*-CWS. In the last decade, Dr. Azuma worked extensively to develop synthetic immunopotentiators and applications for immunoprophylaxis of bacterial and viral infections.

Since its formation, Dr. Azuma has been a member of Japanese Tuberculosis Panel and submitted papers with Dr. Yamamura for almost every Joint Conference. Dr. Azuma became Chairman of the Japanese Tuberculosis Panel in 1988.

Dr. Shigeichi Sunahara
Chairman

Dr. Sunahara had been a member of the Japanese Tuberculosis Panel, and then became chairman after Dr. Iwsaki. Dr. Sunahara had been a director of National Sanatorium Tokyo Hospital, one of the largest sanatoriums in Japan. He conducted "the Research Group of National Sanatoria on Chemotherapy of Tuberculosis" and established criteria to evaluate the efficiency of various regimen of anti-tuberculosis drugs. He also performed excellent studies on the pharmacogenetics of isoniazid metabolism. Dr. Sunahara is a founder of Tokyo National Chest Hospital, School of Rehabilitation, the first college to educate physiotherapists in Japan.

Dr. Sunahara died in June 1988 as a result of cerebral bleeding. His unduly sudden death was an immense sorrow to all those who knew him and admired his achievements in the field of tuberculosis.

Dr. Tatsuro Iwasaki
Chairman

Starting his academic career as a pathologist, Dr. Iwasaki has been, and still is, the most eminent leader in the field of tuberculosis research in Japan. He proposed a classification system of pulmonary tuberculosis according to chest X-ray findings as reflection of patho-histological changes in the lung. This classification system, with minor modifications, is still widely used in Japan. As a director of the Research Institute of Tuberculosis of Japan Anti-Tuberculosis Association, he has been concerned not only in basic research but also in tuberculosis control. His recent two books, entitled "Natural History of Tuberculosis" and "History of Tuberculosis in Japan" (regrettably, in Japanese), evidenced his wide view in the knowledge on tuberculosis.

Dr. Iwasaki's hobby is painting. The cover of the First Five-Year Report of U.S.-Japan Program was designed according to Dr. Iwasaki's motif. After his resignation from the chairmanship of the Tuberculosis Panel, Dr. Iwasaki further devoted himself to US-Japan Program as a member of the Delegation. Dr. Iwasaki, now Emeritus Director of the Research Institute of Tuberculosis, is still teaching not only staff of the Institute but also trainees from developing countries.

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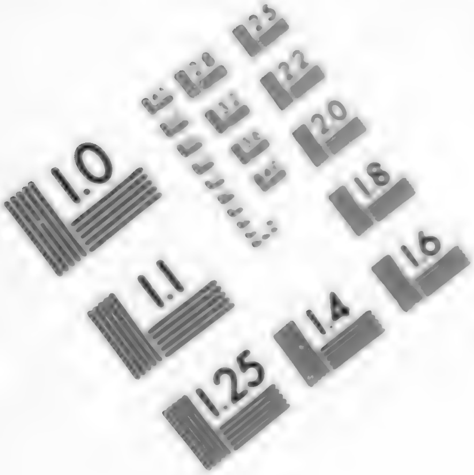

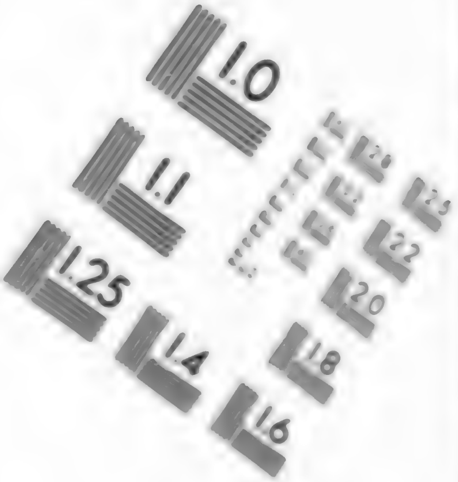

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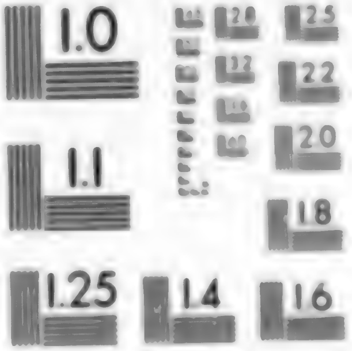

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Dr. Yuichi Yamamura

Chairman

After returning from the navy, Professor Yamamura started his research career in the Department of Organic Chemistry, Faculty of Science, Osaka University and worked as a medical doctor in the National Sanatorium Toneyama Hospital. At that time, he proved, in rabbits, that experimental tuberculosis cavities were produced by the allergic reaction to the cellular components, especially protein fraction of tubercle bacilli, not by living tubercule bacilli. This is not just one of the most important works of experimental tuberculosis but also a pioneer study in the field of cellular immunology. He was appointed as Professor of Medicinal Chemistry, Faculty of Medicine, Kyushu University in 1958 and then he became the Chairman and Professor of Internal Medicine, Osaka University Medical School in 1962. As a professor of medicine, he expanded his research into immunology, hematology, endocrinology, oncology, etc. Especially, he was interested in the biochemical and immunological studies of tubercle bacilli, and he has worked extensively on the application of mycobacterial cell-wall skeleton on cancer immunotherapy. He was elected as a president of Osaka University and stayed in this position from 1980 to 1986.

Since the start of the Program, he was a member of the Tuberculosis Panel and later, Chairman. Subsequently, he was a member of the Japanese Delegation. He has made great contributions to the organization and development of the Program by his broad experience on basic and clinical medicine. He is still active as a scientist, especially in the fields of immunology, and also as a leader of Japanese science.

Dr. Tohru Tokunaga

Chairman

Dr. Tokunaga has worked on the bacteriology of mycobacteria since his graduation from Kyushu University, first at the University and then the NIH, Tokyo. He discovered transfection of mycobacteriophage DNA, liposaccharide phage receptor, and sexual conjugation of mycobacteria, etc. Then his interests turned to the host response against mycobacteria, especially the mode of antitumor action of BCG. As the Director of Department of Tuberculosis of NIH and then of Department of Cellular Immunology, he has conducted research in the fields of microbiology and immunology: for example, differentiation and activation of macrophages, and host response to bacterial DNA.

On the Tuberculosis Panel, his wide experience from molecular genetics to animal experiments were useful for organizing and promoting tuberculosis research. Especially young investigators in this field have been encouraged by Dr. Tokunaga's leadership and friendship.

GUIDELINES

TUBERCULOSIS PANELS
United States–Japan Cooperative Medical Science Program

Tuberculosis research is concerned primarily with projects relating to the pathogenesis and immunological aspects of the disease. Subjects of special interest are immunity and hypersensitivity, the analysis of cell substances of tubercle bacilli which might be related to immunity, and experimental models for the study of tuberculoimmunity:

1. Biology of mycobacteria

a. Fractionation and identification of biologically and immunologically active constituents of tubercle bacilli
b. Genetics and physiology
c. Mechanisms of drug action and development of resistance

2. Pathogenesis of tuberculosis and other mycobacterioses

a. In man and experimental models
b. Host-parasite relationship
c. Environmental and nutritional factors

3. Immunology of tuberculosis

a. Mechanisms of hypersensitivity, anergy, and acquired resistance
b. Immunoglobulin response to infection and immunization
c. Activation of nonspecific effector mechanisms of mycobacterial and other products
d. Adjuvant properties of mycobacterial and other products

4. Immunoprophylaxis

a. Live vaccines
b. Killed vaccines
c. Natural and synthesized mycobacterial fractions

To facilitate these studies, special reagents are made available only for research purposes to qualified investigators: lyophilized PPD tuberculin, characterized mycobacterial cultures of research interest, a culture filtrate reference antigen preparation and polyvalent reference antiserum with which investigators are able to standardize laboratory produced material.

U.S. Tuberculosis Panel

PROGRESS IN PRIORITY AREAS

Tuberculosis research carried out under the aegis of the U.S. Tuberculosis Panel has continued largely along established lines but in addition, a major shift has also been seen over this time reflecting the influence of recent advances in molecular biology and genetics. New techniques have been adapted to studies of virulence and protective antigens associated with *M. tuberculosis* and their role in the development of acquired resistance in the tuberculous host. Substantial advances have been made in three main areas; namely, the chemistry of the glycolipid antigens associated with the cell-wall, the nature of the cellular interactions involved in the protective immune response and the development of recombinant vaccines and monoclonal antibodies for immunizing, taxonomic, and diagnostic purposes. Much of the impetus for these studies stems from the emerging AIDS epidemic in this country, which is associated with disseminated tuberculous and nontuberculous disease, much of it untreatable by conventional chemotherapy. However, there has also been the realization that current measures being used for the control and eradication of tuberculosis worldwide simply are not doing the job and that new, innovative reagents and procedures are urgently required. This has led to increased support by the NIH in this area which should lead to further advances in the near future.

A comparison of the major topics of interest in tuberculosis research only 5 years ago with those currently most active reveals several exciting trends with implications for future directions being taken by researchers over the next 5 years. The major thrusts now seem to in the areas of the molecular mediators of immune activation, gene cloning, hybridoma formation, and recombinant vaccine production. However, there have also been substantial advances in our understanding of the chemistry of glycolipid and other polysaccharide antigens associated with cell-wall antigens useful in the taxonomy and serotyping of various non-tuberculous mycobacterial species. In addition, there have been important advances in our understanding of the immunological role played by several protein antigens which seem to be related to the heat-shock proteins produced by a variety of organisms exposed to a variety of environmental stresses. These proteins are somehow involved in the induction of the immune response by the infected host. Some of these proteins (and their genes) bear a striking degree of homology with "heat-shock" proteins although the reason for their unexpected biological activity within the susceptible host remains unclear. Finally, there has been an increasing degree of sophistication in studies of the molecular mediators of the cellular immune response, involving various lymphokines and monokines responsible for the activation and later, the modulation of the protective response. This increasingly molecular approach to tuberculosis research marks a trend which can be expected to accelerate during the next 5-year period.

SIGNIFICANT ACCOMPLISHMENTS

The major thrust of this program has concentrated on reaching a better understanding of the pathogenesis of all forms of mycobacterial disease (excluding leprosy), as well as determining the means by which the host defenses prevent (or at least limit) the further development of progressive disease. Many of these studies have involved the cellular mediators of the immune response, especially those entering the infected lungs of vaccinated vs. the normal host. In addition, the role played by various lymphokines (especially MIF and IFN) during macrophage activation within the developing tubercle has been examined in some detail. Such studies have established the fact that immunologically activated macrophages are capable of preventing the further growth and spread of virulent tubercle bacilli *in vivo* and thus protecting the host against the lethal effects of the tuberculous challenge. These same cells seem to be also involved in the tuberculin skin test and in the expression of the non-specific antitumor response induced by BCG vaccine. The therapeutic value of BCG cell-walls, trehalose dimycolate (cord factor) and a variety of its analogues has been tested for their potential in the treatment and prevention of pulmonary tuberculosis as well as anti-tumor agents. Extensive chemical studies of the structure and synthesis of these unique and highly active compounds have been carried out, together with studies of a number of sulfatides which appear to be involved in the survival of some virulent strains of tubercle bacilli within the phagolyosome of the activated macrophage. Finally, there have been extended studies into the isolation, chemistry, and biological activity of various tuberculopeptides in an attempt to determine the "specific" epitope associated with the tuberculin molecule which might identify those individuals who are actively infected with *M. tuberculosis* compared to those vaccinated with BCG or colonized with the opportunistic atypical mycobacteria. Paradoxically, the use of increasingly sophisticated electrophoretic and chromatographic techniques to purify the tuberculin merely served to increase the heterogenicity of the product and despite continued attempts to define the specific allergenic moiety, tuberculin continues to cross-react with the corresponding products isolated from various non-tuberculous mycobacteria. The dream of developing a synthetic specific tuberculin capable of identifying patients with active tuberculous disease remains as elusive as ever.

The adaptation of gene cloning and cell hybridization techniques to tuberculosis research has resulted in a number of dramatic advances in the past 5 years. Initially, the development of monoclonal antibodies against specific mycobacterial antigens was exploited for their obvious taxonomic potential as well as being useful epidemiological tools. They also provided the means for isolating these protein antigens produced in large amounts by recombinat cells *in vitro*. At the same time, specific T-cell clones were developed giving an increasing insight into the reactive epitopes associated with specific immunogens produced *in vivo* by this pathogen. Identification of the genetic libraries responsible for the production of these specific immunogenic proteins which were then cloned into *E. coli* K12 proven to be surprisingly simple, providing a ready source of pure protein antigen for further laboratory testing. However, transferring these genes into other mycobacterial species such as BCG or *M. smegmatis* has proven to be somewhat more of a technical challenge only recently solved by means of a newly developed "phasmid" vector and electroporation. Development of fully protective recombinant vaccine for human use may be still some time away, however, since there is abundant evidence that avirulent *E. coli* or *M. smegmatis* recombinants are unlikely to induce a cellular immunity unless suspended in a Freund-type adjuvant which is too toxic for human use

MAJOR ACHIEVEMENTS

a. Development of quantitative assays for measuring the growth of tubercle bacilli in vaccinated vs. normal mice and guinea pigs using a biologically relevant aerogenic challenge.

b Demonstration of the role of T cells in DTH vs. CMI responses within the anergic infected host. Identification of the mediators of macrophage activation by antigenically stimulated T cell subsets.

c. Development and refinement of a serotyping system for non-tuberculous mycobacteria and the establishment of a Type Culture Collection with carefully documented, standard strains of mycobacteria of pathogenic importance.

d. Isolation and identification of various mycobacterial glycolipid and protein antigens of importance in taxonomy and pathogenesis studies.

e. Development of monoclonal antibodies specific for various mycobacterial antigens.

f. Identification of the gene (or genes) responsible for various mycobacterial "virulence" and "protective" antigens and/or immunogens and the development of recombinant vaccines for possible human use.

FUTURE GOALS

Pulmonary tuberculosis still ranks as one of the major infectious diseases in the world today. The World Health Organization estimates that as many as three million victims die from this disease annually. With the currently available technology for immunization (Bacillus Calmette-Guerin vaccine), realistic prospects of eradicating tuberculosis worldwide are meager. Indeed, the dismal failure of the recent BCG vaccination field trial sponsored by WHO in the Chingleput region of South India, has brought a number of calls for a reappraisal of the whole immunization program. Subsequently, a new, ambitious and highly sophisticated research program into the immunology of tuberculosis has recently been launched. This reaffirms the original commitment of the U.S.-Japan Cooperative Medical Sciences Program set up to clarify such problems and which complements other current approaches relevant to elucidating the pathogenesis of this important, life-threatening human disease. Throughout the tenure of the U.S.-Japan Tuberculosis Program, attempts to understand and manipulate the two phenomena, immunology and pathogenesis, have remained major research objectives. With the emergence of the AIDS epidemic in the United States and now in Japan this work becomes even more important and insistent. The occurrence of life-threatening systemic *M. avium*-complex infections in a substantial proportion of the AIDS population makes studies of immunotherapy in the immunodepleted host a prime research objective. In addition, the high degree of drug-resistance expressed by these opportunistic organisms against most first-line anti-tuberculous drugs gives impetus to basic studies of the underlying resistance mechanisms within these organisms, as well as for the development of new drugs and treatment regimens which can provide more effective treatments for these unfortunate patients.

PROJECTS SUPPORTED BY THE PROGRAM

GRANTS

Investigator and Institution	*Title*
Peter F. Barnes University of Southern California	Immunologic Significance of cell Walls in Tuberculosis
Willem H. Boom Case Western Reserve University	CD4+ T-Cells Heterogeneity in *M. tuberculosis* Infection
Willem H. Boom Brigham and Women's Hospital	Protective Antigens of *Mycobacterium tuberculosis*
Patrick J. Brennan Colorado State University	Defined Native Antigens and Immunity to Tuberculosis
Anthony M. Coates University of London	Improved Vaccinia Recombinants for Tuberculosis Vaccine

Frank M. Collins Trudeau Institute, Inc.	Protective Epitopes of BCG Protective Sensitins
Jack T. Crawford University of Arkansas Med Scis	*M. tuberculosis* Genome Structure and Repeated Sequences
Thomas Daniel Case Western Reserve University	Prevention/Diagnosis of TB in HIV-Infected Patients
Thomas Daniel Case Western Reserve University	Specific Mycobacterial Antigens
Jerrold J. Ellner Case Western Reserve University	Immunosuppression in Mycobacterial Diseases
Jerrold J. Ellner Case Western Reserve University	Protective/Diagnostic Recombinant Mycobacterial Antigens
Mayer B. Goren National Jewish Ctr for Immunology/Res	Surface Chemistry of Mycobacteria
Marcus A. Horwitz University of California Los Angeles	Complement and Opportunistic Mycobacteria in AIDS
Robert N. Husson Whitehead Institution for Biomedical Research	Genes and Antigens of *M. tuberculosis* Relative to Immunity
William R. Jacobs Yeshiva University	Diagnosis of Tuberculosis by Recombinant Shuttle Plasmid
Richard A. Lerner Scripps Clinic and Research Foundation	Reactivity of Molecularly Defined Mycobacterial Antigens
David N. McMurray Texas Agri and Mech Univ College	Protein and Zinc Deficiency and Vaccine Efficacy
Quentin N. Myrvik Wake Forest University	Interaction of Alveolar Macrophages with Mycobacteria
Ramchandra G. Navalkar Morehouse School of Medicine	Reactivity of Molecularly Defined Mycobacterial Antigens
Thomas M. Shinnick Scripps Clinic and Research Foundation	Reactivity of Molecularly Defined Mycobacterial Antigens
Donald W. Smith University of Wisconsin Madison	Virulence of *M. tuberculosis* Pathogenetic Significance
Robert S. Wallis Case Western Reserve University	Hybridoma Selection—Recombinant Mycobacterial Antigens
Donald L. Wise Northeastern University	Tuberculosis Chemotherapy with Biodegradable Polymers

CONTRACTS

Sanders J. Sevall Specialty Laboratories, Inc.	Diagnosis of Mycobacterial Diseases by Polymerase Chain Reaction

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Japanese Tuberculosis Panel

BROAD GOALS

The guidelines for the Tuberculosis Panels were last revised in 1978. Research activities of the Panel were concerned primarily with projects related to pathogenesis and immunological aspects of tuberculosis. A specific listing of the current goals of the Program is found at the beginning of this section.

PROGRESS IN PRIORITY AREAS

Biology of mycobacteria

Fine structure of mycobacteria: Ultrafine structures of mycobacteria were analyzed with scanning electron microscopy and energy dispersive X-ray analyzer (EDA). Chromosomes were prepared from spheroplasts of various species of mycobacteria and observed under electron microscopy. Electron-dense granules of mycobacteria were analyzed with EDA and shown to contain high concentrations of phosphorus and smaller amounts of potassium.

Isoniazid-resistance: Tubercle bacilli rendered resistant to isoniazid lose their catalase and peroxidase activities as well as virulence. Heme synthetic activity of such strains do not significantly differ from isoniazid-susceptible strains, the resistant strain could not synthesize apo-catalase. Gene coding apo-catalase may be relevant with respect to resistance or virulence.

Mycobacterial lipids

Alpha-mycolic acids were isolated from rapidly and slowly growing mycobacteria and analyzed with gas-chromatography/mass-spectrometry (GC/MS). Each mycobacterial species showed characteristic profiles of alpha-mycolic acid molecular species. Mycobacteria could be classified into 8 groups, according to total numbers of carbon, unsaturated bonds, and length of branched chains. Among glycolipids of mycobacteria, those with glucose- or trehalose showed strong granuloma-forming activities, especially when given in a form of liposome.

New glycolipids, trehalose-2,3,6'-trimycolate and highly acylated trehalose were isolated and characterized.

Antigenic proteins of mycobacteria

During the last 5 years, gene technological procedures were widely applied to isolate and characterize antigenic proteins of tubercle bacilli.

Genes to encode immunogenic proteins of *Mycobacterium bovis*, BCG, such as MPB 57, MPB 64, MPB 70 and α antigen, were cloned and their nucleotide sequence was characterized. The gene to encode 15 kDa protein of *Mycobacterium tuberculosis*, Aoyama B was also cloned and sequenced. These mycobacterial genes were expressed in *Escherichia coli*.

Octa- and hexadeca-peptide, which were reported as the active center of tuberculin active protein, were chemically synthesized. These synthetic peptides did not show skin-reactivity.

In order to advance gene technology for mycobacteria, shuttle vector, which functions as a plasmid in *Escherichia coli* and as a bacteriophage in mycobacteria, was developed. Introduction of this shuttle vector into mycobacterial cells was facilitated by electroporation.

Monoclonal antibodies specific for *Mycobacterium tuberculosis* complex and also those which could differentiate between *Mycobacterium avium* and *Mycobacterium intracellulare* were prepared by hybridoma techniques.

These studies may be milestones for the development of species-specific skin-reactive antigens useful for diagnosis and also for development of better protective vaccines than BCG.

Studies on ribosomes of mycobacteria

The number of rRNA genes of various species of genus *Mycobacterium* was analyzed in relation to the speed of growth of respective species. Slowly growing species, such as *Mycobacterium bovis* BCG and *Mycobacterium lepraemurium*, showed just one rRNA gene. Complete nucleotide sequence of the 16S rRNA gene of *Mycobacterium bovis* BCG was clarified. The primary structures of 5S ribosomal RNA of various species of *Mycobacterium* were compared in order to better clarify evolutionary shifts.

Granuloma formation in tuberculosis

T cell line reactive to tuberculin-active peptide (TAP) was established from the lymphnode of nu/+ mice immunized with *Mycobacterium tuberculosis*. This T cell line produced hepatic and splenic granuloma by systemic adoptive transfer to nu/nu mice infected with BCG. The same T cell induced the delayed-type footpad response by local passive transfer. Surface phenotype of the anti-TAP T cell was Thy 1.2$^+$, Lyt 1.2$^+$, L3T4$^+$ and Lyt 2$^-$.

Granulomas were produced by intravenous injection of mycobacterial glycolipids containing glucose or trehalose in their moiety, especially when administered in a form of liposome.

T cells in immunity in tuberculosis

Various T cell clones were shown to be relevant to various aspects of immunity in tuberculosis. A clone, reactive to tuberculin-active peptide (TAP), showed surface phenotype of Thy 1.2$^+$, Lyt 1.2$^+$, L3T4$^+$ and Lyt 2$^-$. It conferred granuloma-forming ability to nude mice infected with BCG. Another T cell clone was specific to PPD, and developed delayed-type hypersensitive reaction when injected into footpad with PPD. Intracellular multiplication of BCG was suppressed within syngeneic macrophages when this T cell clone was added to an *in vitro* culture system. This cloned T cell was effective in preventing an *in vivo* local infection as well.

A human T cell clone, specifically reactive to PPD, was established from peripheral blood mononuclear cells of a tuberculin-positive healthy adult. This cloned T cell was designed OKT3$^+$, 4$^+$, 8$^-$, Ia$^+$, so it belonged to helper/inducer T cell lineage, and produced MIF in the presence of PPD and antigen-presenting cells.

T cells mediating delayed hypersensitive reaction and/or acquired cellular resistance to *Listeria monocytogenes* could be induced *in vitro*. When normal spleen cells were cultured in the presence of live *Listeria*, these cells conferred DTH and acquired cellular resistance. Spleen cells cultured with killed *Listeria* mediated only DTH, but did not confer acquired cellular resistance.

These studies may be very helpful in understanding the relationship between delayed-type hypersensitivity and protection immunity in tuberculosis. They may shed light on development of a new protective vaccine that does not induce DTH.

Suppression of delayed-type hypersensitivity

There are two types of inbred strains of mice with respect to responsiveness to PPD after BCG sensitization, namely, high and low responders. The underlying mechanism of low responsiveness appeared due to the induction of suppressor T cells. Macrophage clones were established in order to clarify the role of the macrophage in induction of suppressor T cells. An I-A positive/I-J positive clone functioned as an antigen-presenting cell in the *in vitro* induction of suppressor T cells. Another Ia negative clone did not induce the suppressor T cells. Treatment of the former clone with anti-I-J^k antiserum abolished the ability to induce the suppressor T cells. It appears that the precursor of the suppressor T cell recognized antigen-determinants of BCG presented on the I-A/I-J positive antigen presenting cell in association with the I-J antigen. It then differentiated into suppressor cells.

Native resistance against infection with *Mycobacterium intacellulare* in mice was determined genetically. In the case of susceptible strains of mice, the infection progressed gradually until the death of animals. But DTH to PPD2Dintra remained negative or very weak throughout the course of the infection. During the early phase of *Mycobacterium intracellulare*-infection, a suppressor T cell suppressed the induction of DTH. At a later phase of the infection another type of suppressor T cell that suppressed both induction and expression of DTH was induced. Both types of suppressor T cell were Thy-1 positive and Lyt 2 negative. The former was I-J negative, and the latter was I-J positive.

Suppressor T cells were not the only cells which showed suppressive effects. Circulating monocytes taken from patients with extensive pulmonary tuberculosis suppressed PPD-induced lymphocyte blastogenesis. In such patients, the production of IL-2 and surface expression of the IL-2 receptor by T cells was depressed. Such immunosuppression is linked to exaggerated production of IL-1 by macrophages.

Deoxyribonucleic acid fiber released from a spheroplast of *Mycobacterium smegmatis*. A double-stranded DNA fiber spreads continuously around a collapsed bacterial cell. (x 30,000)

Adjuvant properties of mycobacterial and other products

Various analogues and/or derivatives of muramyl-dipeptide (MDP) and cord-factor were chemically synthesized. Their adjuvant and anti-tumor activities were compared. Some showed potent adjuvant activities and/or anti-tumor activities.

Lymphokines

IL-5, a new lymphokine, was first demonstrated in culture supernatant of *Mycobacterium tuberculosis*-primed T cells derived from spleens of mice stimulated with PPD. IL-5 acts on activated B cells as a B cell growth and differentiation factor. Further, IL-6 was identified as a lymphokine that is involved in the final differentiation of activated B cells into antibody-forming cells. It also induced the differentiation of myeloid leukemia cells into mature macrophage-like cells.

These lymphokines were purified and were prepared by recombinant DNA technology.

SIGNIFICANT ACCOMPLISHMENTS

Immunogenetics of Tuberculin Skin Reaction

The tuberculin reaction is a typical delayed-type hypersensitive reaction, and has been widely applied clinically and epidemiologically to detect the infection by mycobacteria, including human tubercle bacilli. The degree of tuberculin skin reaction is influenced by the strength of the infection and by various other factors but is fundamentally under the genetic control. Immunogenetic studies on tuberculin responsiveness have progressed remarkably during these 25 years.

Various inbred strains of mice can be classified into two categories according to the responsiveness to tuberculin, PPD, after sensitization with *Mycobacterium bovis*, BCG. These inbred-strain mice such as SWM/Ms, SJU/J, SJA/9 and ICR are high responders, and C3H/He, CBA, DBA/2, BALB/C, and A/SW are low responders. Analyses of tuberculin responsiveness of F_1, F_2 and back-cross hybrids between high-responder SWM/Ms and low-responder C3H/He suggested that the responsiveness is controlled by an allele in which high responsiveness is dominant over low responsiveness and that this gene links to neither H-2, coat color, nor Igh allotype.

The mechanism of low responsiveness to PPD also has been clarified. Two weeks after subcutaneous injection of live BCG, C3H/He mice showed very weak (0.35 ± 0.11mm) footpad reactions to 10 ug of PPD, in comparison to strong positive reactions (0.99 ± 0.21mm) of SWM/Ms. Such low responsiveness of C3H/He mice recovered to the same level of responsiveness as SWM/Ms by pretreatment of C3H/He mice with cyclophosphamide before BCG sensitization. When spleen cells obtained from cyclophosphamide-untreated C3H/He mice were passively transferred into cyclophosphamide-treated C3H/He mice, recipient mice showed very weak tuberculin footpad reactions. This suppressive effect of transferred cells was abolished by *in vitro* treatment of the cells with anti-Thyl 1, anti-Lyt 1, or anti-Lyt 2 monoclonal antibody plus complement but was not influenced by the treatment with anti-I-J^k antibody plus complement. These results strongly suggested the existence of suppressor cells of T cell nature in low responders. Such suppressor T cells could be induced *in vitro* by incubating spleen cells obtained from nonsensitized C3H/He mice in the presence of PPD. For the induction of the suppressor T cells *in vitro*, macrophages that coincided with T cells in respect to I-J^k was essential.

An important unsolved problem is the relationship between the gene which controls responsiveness to tuberculin and the gene(s) which may control the ability to acquire protection-immunity against infection with virulent *Mycobacterium tuberculosis*. It has been already shown that natural resistance of mice against mycobacterial infection is controlled by a single gene, designated as *Bcg* and localized on chromosome 1. The gene which controls responsiveness to tuberculin is clearly distinct from *Bcg* gene. Data now available suggested that the responsiveness to tuberculin might correlate with the ability to acquire the protective immunity. Studies on this problem may give a clue to elucidate a correlation between tuberculin allergy and protective immunity in tuberculosis, and also lead to development of a new protective vaccine without giving tuberculin positivity.

Adjuvant Activity of Tubercle Bacilli and BRM

It is well-known that tubercle bacilli are potent immunological adjuvants. Cell-wall fraction of tubercle bacilli is as active as whole cells. During these 25 years, studies on the active principle for adjuvant activity of tubercle bacilli have progressed remarkably, and finally the minimal structural requirement for adjuvant activity has been established.

The cell wall of tubercle bacilli is composed of lipopolysaccharide moiety and mucopeptide moiety. Adjuvant activity is exclusively depended on the latter part of the mycobacterial cell wall.

By degradation studies and synthesis of plausible candidates, the N-acetylmuramyl-L-alanyl-D-isoglutamine portion of mucopeptide has been shown to be the adjuvant-active principle of tubercle bacilli.

N-acetylmuramyl-L-alanyl-D-isoglutamine (MDP) shows various types of immunoadjuvant activities. Biological activities of MDP include enhancement of antibody production, induction of delayed-type hypersensitivity, induction of helper T cells and activation of macrophages.

Various analogues and derivatives of MDP have been synthesized, and their biological activities compared with MDP. Some of the derivatives, such as 6-0-mycoloyl-MDP and quinonyl-MDP-66, show antitumor activity in addition to the adjuvants activities of MDP.

These studies, starting from the study on adjuvant activity of tubercle bacilli, have led to the modern concept of "biological response modifier (BRM)" and some BRMs have a possibility to be used as immunostimulants and in the immunotherapeutics of cancer.

FUTURE GOALS

- To characterize the pathogenicity or virulence factor of tubercle bacilli.
- To obtain species-specific antigen for skin-tests which will differentiate *Mycobacterium tuberculosis* from other mycobacteria. To develop sensitive and specific diagnostic methods using serological tests or DNA-hybridization.
- To clarify the mode of action of and the mechanism of resistance to anti-tuberculosis drugs.
- To clarify the mechanism of macrophage activation.
- To analyze the dynamics and roles of respective subsets of T cells in protective immunity and delayed-type hypersensitivity reactions in tuberculosis.
- To clarify the immunogenetic factors which determine the native and acquired resistance to mycobacterial infection.
- To develop new improved protective vaccines other than BCG.
- To obtain large quantities of specific antigen(s) by gene technology.
- To obtain clinically applicable adjuvant substance or biological response modifiers.

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Joint Scientific Conference

TUBERCULOSIS PANELS

Date: August 14–16, 1985

Place: NIH, Bethesda, Maryland

Summary: The 20th Conference was held in Bethesda, Maryland, at the National Institutes of Health. On August 14, a joint symposium on leprosy and tuberculosis was held, and on August 15 and 16, papers by members and guests of the Tuberculosis Panels were presented. The subject of the joint symposium was "Recent Advances in Immunology and the Immunology of Leprosy and Tuberculosis." Nine representative papers were presented by the panel members and guests. The Conference on Tuberculosis was organized into four separate sessions including: the structure and function of cell-wall components, resistance and modulation in the immune response, antigen specificity and function, and antibodies and the immunosuppressed host. A total of 27 papers were presented.

Date: September 24 and 25, 1986

Place: International Hotel, Osaka, Japan

Summary: The 21st Conference was held in Osaka, Japan, at the International Hotel. On September 26, a joint symposium on leprosy and tuberculosis was held, and on September 24 and 25, papers by members and guests of the Tuberculosis Panels were presented. The subject of the joint symposium was "Molecular Biology of Mycobacteria." Seven representative papers were presented by the panel members and guests. The Conference on Tuberculosis was organized into four separate sessions including: anti-tuberculosis drugs and mycobacterial antigens; mycobacterial lipids and macrophages; T-cell clones and suppression; and protective immunity and the role of a cell. A total of 21 papers were presented.

Date: July 20 and 22, 1987

Place: NIH, Bethesda, Maryland

Summary: The 22d Conference was held in Bethesda, Maryland, at the National Institutes of Health. On July 21, a joint symposium on leprosy and tuberculosis was held, and on July 20 and 22, papers by members and guests of the Tuberculosis Panels were presented. The subject of the joint symposium was "Recent Advances in Immunology and the Immunology of Leprosy and Tuberculosis." Eight representative papers were presented by the panel members and guests. The Conference on Tuberculosis was organized into four separate sessions covering such subjects as: macrophages; species identification and cell-mediated immunity; antigens and gene expression; and adjuvants. A total of 22 papers were presented.

Date: July 27 and 29, 1988

Place: Nara University, Nara, Japan

Summary: The 23d Conference was held in Nara, Japan, at the Nara University. On July 28, a joint symposium on leprosy and tuberculosis was held, and on July 27 and 29, papers by members and guests of the Tuberculosis Panels were presented. The subject of the joint symposium was "Stress Proteins and Antibody Responses to T-dependent Antigens." Seven representative papers were presented by the panel members and guests. The Conference on Tuberculosis was organized into four separate sessions covering subjects such as: DNA probes to identify tuberculosis; immune mechanisms; the T cell response; and suppressor cell induction. A total of 21 papers were presented.

Date: August 23 and 25, 1989

Place: Town & Country Hotel, San Diego, California

Summary: The 24th Conference was held in San Diego, California, at the Town & Country Hotel. On August 24, a joint symposium on leprosy and tuberculosis was held, and on August 23 and 25, papers by members and guests of the Tuberculosis Panels were presented. The subject of the joint symposium was "Specific Detection of Mycobacteria and Vaccine Development." Nine representative papers were presented by the panel members and guests. The Conference on Tuberculosis was organized into four separate sessions covering subjects such as: immune response to stress proteins; PPD-reactive T cells; proliferation of macrophages; and cell wall of mycobacteria. A total of 20 papers were presented.

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Profiles

U.S. VIRAL DISEASES PANEL

Dr. Frederick A. Murphy
Chairman (1987–Present)

Dr. Murphy has been Director, Center for Infectious Diseases, CDC since July 1987. He served as Director of the Division of Viral Diseases, CID, from 1984–1987. Dr. Murphy was the Associate Dean and Professor of Microbiology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, from 1978–1983. He served as Chief, Viral Pathology Branch, CDC, 1964–1978. In 1970, he was an honorary fellow at the John Curtin School of Medical Research, Australian National University. He was Chairman, Program Committee, Fifth International Congress for Virology, 1981, and was Chairman of the Virology Division, International Union of Microbiological Societies from 1981–1984. He was one of the organizers of the American Society for Virology. He is on the editorial board of nine journals. Dr. Murphy received the B.S. and D.V.M. degrees from Cornell University and a Ph.D. in immunology from the University of California. He has served as Chairman of U.S. Viral Diseases Panel of the USJCMSP since 1987 and has been a member of the Panel since 1986.

Dr. Thomas M. Yuill
Chairman (1982–1987)

Doctor Yuill was born in Berkeley, California in 1937, and grew up in rural Maryland. He received his B.S. degree from Utah State University in Wildlife Management, and his M.S. and Ph.D. degrees from the University of Wisconsin-Madison in Veterinary Science and Wildlife Ecology, with emphasis in the epizootiology of infectious diseases of domestic and wild animals. After receiving his Ph.D., he held research positions in the Department of Virus Diseases, Walter Reed Army Institute of Research, and in the Virology Department of the SEATO Medical Research Laboratory, Bangkok, Thailand. He joined the faculty of the University of Wisconsin-Madison as an assistant professor in 1968, with joint appointments in the Departments of Veterinary Science and of Wildlife Ecology, developing research and graduate training programs in epizootiology of infectious diseases of wildlife and domestic animals in Wisconsin and Latin America. He became professor and chairman of the Department of Veterinary Science in 1979. He was appointed Associate Dean for Research and Graduate Training in the new University of Wisconsin School of Veterinary Medicine and an assistant director in the Agricultural Experiment Station in 1982. Although primarily involved in academic administration, Dr. Yuill has continued his research and graduate training activities with projects in the United States and in Central America. Doctor Yuill has been active in a variety of scientific organizations. He has been active in the Organization for Tropical Studies (OTS) since 1971. He has been on OTS Executive Committee and has also served as president for 5 years. He has served as treasurer and president of the Wildlife Disease Association, which awarded him its Distinguished Service Award in 1989. He is currently serving as an elected member of the Board of Directors of the Center for Tropical Agriculture Research and Teaching (CATIE) located in Central America, with its major base in Turrialba, Costa Rica. Doctor Yuill has served as a consultant on wildlife diseases to the states of Wisconsin and North Dakota and the U.S. Environmental Protection Agency, on arthropod-borne diseases to the U.S. National Institutes of Health, and on higher education in the agricultural sciences to the U.S. Agency for International Development, mainly in India. He has authored over 100 scientific papers and book chapters. Dr. Yuill was a member of the Viral Diseases Panel of the US–JCMSP from 1976 to 1987 and chaired the Panel from 1982 to 1987.

Dr. Edwin H. Lennette

Chairman

Dr. Lennette received his B.S., M.D., and Ph.D. degrees from the University of Chicago where he remained as an instructor in microbiology until accepting an appointment at Washington University School of Medicine in St. Louis, Missouri. In 1939, he moved to the Rockefeller Institute where he worked on the newly discovered influenza virus. After working on yellow fever in Brazil, he returned to the U.S. to work on viral respiratory diseases in the Berkeley laboratory of the California State Department of Public Health. There he developed the first civilian viral diagnostic laboratory in the U.S. Dr. Lennette established, and became director of, the Viral and Rickettsial Disease Laboratory of the California Department of Health Services where he remained until his retirement in 1978.

Dr. Lennette has served the virology community well through his membership in numerous scientific and medical societies and as a consultant for many prestigious organizations in the United States and as an Expert Consultant on Viral and Rickettsial Diseases for the World Health Organization. Currently, Dr. Lennette is the President of the California Public Health Foundation in Berkeley, California.

Dr. William F. Scherer

Chairman (1978–1980)

A past president of the American Society of Tropical Medicine, Dr. William F. Scherer was noted for his research in virology and was an authority on arboviruses. He was born in Buffalo, New York, and received his M.D. from the University of Rochester in 1947. After interning in internal medicine at Barnes Hospital, St. Louis, he returned to Rochester for a pathology internship at Strong Memorial Hospital, then went to Vanderbilt University Hospital as an assistant resident in internal medicine. His interest in viruses grew with his appointment as an associate professor at the University of Minnesota Medical School, and as National Research Council Fellow in virology.

Dr. Scherer was only 27 years old when he published a series of papers on the propagation of polio viruses *in vitro* that established that this virus could be grown in extra-neural tissues. Techniques that he developed contributed to reducing the complex phenomenon of animal-virus infection to a simpler in vitro system. For this and other valuable research, Dr. Scherer received the Theobald Smith Award of the American Association for the Advancement of Science in 1959. Dr. Scherer's subsequent research focused on the encephalitis virus, much of which was carried out during his tenure at Cornell Medical School.

Dr. Scherer was honored by appointment to a number of prestigious committees in the field of his expertise. He served on the Viral Diseases Panel from 1974 to 1980 and as its chairman from 1978–1980. Dr. Scherer died in 1982.

Dr. Fred M. Davenport

Chairman (1969–1973)

A native of Pennsylvania, Dr. Fred M. Davenport completed undergraduate studies at Columbia University in 1936, entering that same institution's College of Physicians and Surgeons and earning the M.D. degree in 1940.

Dr. Davenport's training was interrupted by World War II during which he served as a malariologist in the Army Medical Corps. In 1946, Dr. Davenport returned to Columbia University to continue work toward the Sc. Med. D. degree and to serve as Assistant Instructor in Pathology. In 1947, he was awarded a National Research Council Fellowship in Virus Diseases which enabled him to spend 2 years in additional postdoctoral training at the Rockefeller Institute for Medical Research working under Dr. F. L. Horsfall on serological research with influenza viruses.

Dr. Davenport joined the faculty of the School of Public Health in 1949 as a Research Associate in Epidemiology to collaborate with Dr. Thomas Francis Jr., in research on the virology and epidemiology of influenza and other virus diseases. He remained in that institution, serving as Professor in the Departments of Epidemiology and Internal Medicine and Chairman of the Department of Epidemiology from 1969 to 1976.

Dr. Davenport's research on virus diseases and especially on the problem of the control and prevention of epidemic influenza has placed him at the forefront of authorities whose advice was sought at national and international levels. He was an Associate Member of the Armed Forces Epidemiologic Board and served as Director of its Commission on Influenza from 1955 to 1971. Between 1969 and 1973, he served as Chairman of the Viral Diseases Panel. Dr. Davenport was a member of the "blue ribbon panel" of experts who met at the White House in March, 1976, to advise former President Gerald R. Ford on the nationwide swine influenza immunization and consultant to organizations such as the Food and Drug Administration and the World Health Organization.

JAPANESE VIRAL DISEASES PANEL

Dr. Tasuke Konno

Chairman (1989–)

Dr. Tasuke Konno was originally a pediatrician and not a virologist. He was, in fact, known as the only really reliable pediatrician of the entire Pediatrics staff at Tohoku University. Thus, in spite of a reputation for absent-mindedness, he was highly valued by patients for his ability to both diagnose and treat the whole gamut of childhood ailments. Since 1974, when rotavirus was discovered, he has conducted a great many elegant and fascinating virus studies. With his benign and disarming smile, his mild manners and behavior, he has been able to win the confidence of many of Sendai's foreign students, whose lives have been made a great deal easier and more comfortable thanks to the help of Dr. Konno and his wife.

Akira Oya

Chairman (1985–1989)

Dr. Oya, director of the National Institute of Health in Tokyo, is an outstanding leader not only within the Institute but also in the fields of epidemiology and preventive medicine in Japan. For a long time, his work at the Department of Virology and Rickettsiology has focused on the natural lives of arboviruses and influenza viruses and on improvement of the vaccines for Japanese encephalitis and flu. In the studies for which he is best known, Dr. Oya established a network system to preestimate the prevalence of Japanese encephalitis during the summer each year by assaying the porcine IgM-IgG antibody rise in epidemic areas. Such surveillance work together with a highly purified vaccine has contributed to the marked diminution of the number of patients suffering from Japanese encephalitis.

A native of Tokyo, Dr. Oya enjoys fishing by the ocean and also talking in detail about the fish he has caught to people at the lab.

Dr. Hideo Fukumi

Chairman

A quiet, not to say taciturn man, Dr. Fukumi sometimes gives visitors an impression of inaccessibility. However, those who know him better testify to his real fondness for company. This is proved, they say, by his involvement with baseball, which he not only watched but also played, although not of course, after becoming President of Nagasaki University. He is an enthusiast for drinking, at least at the level of talking about wine, although he has never shown himself to be very adept at the practice.

Dr. Yasuichi Nagano
Chairman

Born in 1906, Dr. Nagano is now over 80 years old. Nevertheless, he is still as active in his research as a young scientist. The original and independent discovery of the “virus inhibitory factor” has made his name well known in virological circles. Not usually talkative, Dr. Nagano can be moved to enthusiasm and loquacity when discussing virology. He is fluent in French, having studied in France in his youth, and has been awarded the order of the *Legion d'honneur*.

Dr. Masatsugu Kanamitsu
Chairman

Dr. M. Kanamitsu was a rather taciturn and quiet man, however, his speech was very clear and cogent. He was very fond of skiing and mountain-climbing. Dr. Kanamitsu was a pioneering researcher in the field of epidemiology of infectious diseases in Japan. He tried to explain various epidemiological phenomena theoretically. By organizing a study group during his chairmanship of the Japanese Panel, he encouraged arbovirus researchers to search for the factor(s) relating to the recent dramatic decline of JE virus infection in both Japan and Korea. He further put into action a long-term plan to clarify the geographic distribution of various arbovirus infections in Southeast Asia by examining antibody prevalence in residents and reservoir animals and collecting vector mosquitoes. His death in the midst of this ambitious plan, at the age of 74 in 1985, was a great loss felt by many investigators. Dr. Kamanitsu was a man who pursued “originality” throughout his life.

Dr. Minoru Matsumoto
Chairman

Dr. Matsumoto has long been renowned among scientists in Japan for his outstandingly quick understanding. Some of his habits of mind, both in life and in the laboratory, he perhaps owes to his father, who had a remarkably broad knowledge of biology. Dr. Matsumoto's important work in the ecology of viral infections was in all likelihood made possible by his profound knowledge of botany, which in turn was the result of his childhood education. He has always had an affinity for English writing, which is why he began publishing in English even before World War II. When he was on the active list in the Virology Department, English novels and essays were his favorite nocturnal reading. Since recovering from a heart attack at the age of 63, he has enjoyed writing poems and doing water colors as well as reading.

Dr. Nakao Ishida
Chairman

Widely known to the northern public as an outstanding figure in the intellectual world, Dr. Ishida has made contributions in a number of fields. As a professor in the Medical School of Tohoku University, he participated in the training of a large number of physicians destined for public service. The field of virology in Japan has also benefited enormously from his activities. In a later stage of his career, he was an active and highly communicative President of Tohoku University and, thus, established a third claim to public esteem. Still engaged in many civic activities, he was made an Honorary Citizen of Sendai in 1989. A leisure time activity, shared by his wife, is the composition of *haiku*.

Dr. Reisaku Kohno
Chairman

Dr. Kohno was a man of great intelligence with a zest for life. He was extremely fond of painting, and always carried a sketchbook with him to record the impressions of his travels. At the same time, he was a keen sportsman and loved skiing and golf. He had an outstanding curiosity about nature, and it was this that brought him to science. He died at the age of 69 in 1985, and has been missed by a multitude of friends in a variety of fields.

Dr. Konosuke Fukai
Chairman (1979–1985)

Professor Fukai was an attractive and generous person as impressed by his physical "giant," and devoted unequivocally to the U.S.-Japan Medical Science Program on Viral Diseases and other international cooperation in Medical Sciences. He was a powerful rugby football player when he was a student, and trained many excellent virologists through his comprehensive scientific attitude, sensitive and enthusiastic practice when he was a Chairman of the Department of Preventive Medicine, Research Institute of Microbial Diseases of Osaka University. He is also skillful in technologies and engineering to invent, utilize, and maintain various scientific instruments for basic and practical virology, especially the electron microscope, ultracentrifuge, and electrophoresis. He has been a pioneer in basic virology in the discovery of a phenomenon later called "von Magnus phenomenon," as well as a practical virologist to develop influenza, Japanese encephalitis, and other major viral vaccines. He is still active as the Chairman of the Research Foundation for Microbial Diseases of Osaka University.

GUIDELINES

VIRAL DISEASES PANELS
United States-Japan Cooperative Medical Science Program

Rabies

Basic, mission-oriented, or applied studies including control measures, early and late diagnostic techniques, virus reservoirs, and prophylactic procedures.

Dengue

Studies of the epidemiology, early confirmatory diagnosis, and pathogenesis of hemorrhagic fever. Development of vaccines using selected viral structural components as the primary antigen: basic immunology with respect to classification; biophysical and biochemical studies.

Arboviruses

Studies on the structural elements of arboviruses, including isolation and identification of antigenic components.

Viral Gastroenteritis

Epidemiologic investigations of infant and adult gastroenteritis using virological techniques; diagnostic methods, antigenic characterization (including human and related animal viruses), and studies on host-immune response requisite for vaccine development, epidemiologic surveillance, or control; detection, propagation, assay, and characterization of putative agents for man; and studies of pathogenetic mechanisms and predisposing factors that could lead to improved prophylaxis, therapy, or control.

U.S. Viral Diseases Panel

PROGRESS IN PRIORITY AREAS

The goals of the Viral Diseases Panels have remained unchanged since 1983 and included research on dengue and other arboviruses, viral gastroenteritis, rabies, and hemorrhagic fever with renal syndrome (HFRS). The advent of new biotechnology using recombinant DNA techniques, monoclonal antibodies, and immunologic methodology has revolutionized many aspects of this program of research and caused a virtual explosion of new information about the viruses, their antigens, and new approaches to vaccine development.

Viral Gastroenteritis

Several candidate vaccines for protection of infants and children against rotavirus infections have been developed and are undergoing evaluation. The most advanced of these involve viruses engineered by genetic reassortant technology. The genome of a rhesus rotavirus (RRV) of serotype 3 was reassorted with genomic segments of human rotaviruses by coinfection in cell culture to produce three variants that carried the backbone of 10 segments of the RRV genome plus the genomic segment coding for the VP7 protein (neutralizing antigen) for one of the other three major human serotypes of group A viruses. As univalent vaccines, each of these variants elicited immune responses in children that were 100% protective against disease when the challenge virus was of the same serotype as the immunogen. However, challenge with heterotypic viruses in clinical field trials revealed less and sometimes unsatisfactory protection. A cocktail of all three univalent variants promised to overcome this problem of specificity, but initial trials revealed that not all variants elicited satisfactory levels of antibodies when administered with the other two serotypes. Adjustments to the dosages of each univalent component of the cocktail may rectify this imbalance in immune response. Another candidate rotavirus vaccine employed a bovine rotavirus that had been passaged repeatedly in cell culture. This vaccine was well tolerated and elicited satisfactory antibody responses but was less than fully protective against challenge with homologous or heterologous serotype challenge viruses.

The use of recombinant DNA technology has been applied to rotavirus vaccine development. Specific genes of rotaviruses have been cloned with baculovirus vectors and have expressed rotavirus proteins in cell cultures. After purification, these proteins are being studied for their biological, biochemical, and immunological properties. Nine of the 11 rotavirus gene products have been expressed so far in this system.

The role of specific epitopes of rotaviral antigens in mediating protective immunity against rotavirus infections has been evaluated by a variety of passive transfer studies using the mouse model. Antibodies to viral proteins VP4 and VP7 (both are envelope antigens) will neutralize the virus in cell culture. The protection elicited by VP7 was serotype specific whereas the protection elicited by VP4 was heterotypic, but further studies revealed that VP7 could induce heterotypic protection.

Seroepidemiologic studies using monoclonal antibodies to the four major group A human serotypes of rotaviruses are being conducted in the United States. In Houston, Texas, the dominant serotype varies from year to year but in the North-central U.S. population, viruses that did not react with any of the typing monoclonal antibodies were identified and are being characterized further to determine if they represent new serotypes.

A major advance in the rapid identification of rotavirus serotype was achieved in Japan using a panel of monoclonal antibodies in an ELISA test. This breakthrough in technology allows the typing of rotaviruses directly from stool specimens without isolation and culture and has greatly enhanced vaccine evaluation field studies where endemic challenge viruses are unknown until detected and identified in the cohort. The rapid typing techniques have probably shortened vaccine development by at least 2 years. Collaborations between the Japanese and U.S. investigators in almost every aspect of rotavirus research also has been a major factor in the rapid progress achieved during the past 5 years.

Dengue and Other Arboviruses

The past 5 years have revealed a renaissance in arbovirus research that can be ascribed to the technological advances in molecular virology, especially DNA cloning, sequencing, and expression of vectored genes in mammalian or invertebrate cells. The new technology has reordered the approaches to studies of arboviruses, arbovirus vaccines, pathogenesis, and epidemiology. The techniques were applied first to nonpathogen, prototype viruses like Sindbis and yellow fever 17D and later to human pathogens. As the alpha and flavivirus genomes were sequenced, mapped, and compared for open reading frames and gene products, surprising new information came forth. The nucleotide sequences of the virulent Asibi strain of yellow fever virus has been compared to its attenuated relative, the 17D strain with implications regarding determinants of virulence. Viral strains, species, and genera revealed new relationships never before realized. For example, it was demonstrated that the alphavirus, Western equine encephalitis virus, is the probable natural recombinant of Sindbis virus and eastern equine encephalomyelitis virus. Approximately one-half of the genome is genetically Sindbis and the remainder is eastern. Now the genomes of several flaviviruses including dengue viruses and Japanese encephalitis virus have been sequenced and are revealing information about the nonstructural proteins encoded by the viruses. NS proteins have been largely unstudied because of the difficulty in producing and purifying them. Most recently, an infectious DNA complement of the 17D strain of yellow fever virus has been cloned, allowing for studies on pathogenesis and viral reproduction at the minutest level using point mutation techniques. Similar applications have been extended to studies of the viruses in invertebrate cells and comparing with infections in mammalian cells.

Studies with dengue viruses have utilized these new technologies to great advantage. A segment of the dengue virus type 4 genome containing the genes coding for the envelope glycoprotein E, the pre-membrane protein M, capsid protein C, and the nonstructural proteins NS1 and NS2a has been cloned using a baculovirus vector and expression in invertebrate cell cultures. The proteins in the cell lysates were used to immunize mice and monkeys. Mice challenged intracerebrally with whole virus were completely protected against the lethal effects of the challenge. Cloning with a vaccinia vector achieved similar protection in mice. In monkeys, protection against viremia following challenge was less obvious. Antibodies to NS1 in monkeys were high but antibodies to E were low.

The dengue type 4 genes encoding for E and NS1 were also cloned using vaccinia virus as vector. After infection with vaccinia virus modified by either dengue E or NS1 genes, mice completely survived intracerebral challenge with the whole dengue virus. Mice receiving passive transfer of serum from the immunized mice were also protected against lethal challenges. Thus, dengue proteins E and NS1 are independently protective in mice.

It is obvious that many additional studies will be necessary to develop an effective dengue virus vaccine, but the technology is now available to do so. Nucleotide sequences of dengue types 2, 3, and 4 are, for the most part, already determined.

Although an effective vaccine against Japanese encephalitis is now available in Japan and China, improved vaccines are being considered that will be developed through recombinant DNA technology. Cloning of the entire genome of JE virus has begun and many of the sequences are already known.

Hemorrhagic Fever with Renal Syndrome (HFRS)

Until the virus was isolated and identified about a decade ago, the serologic tools and reagents were not available to diagnose this disease which now appears to occur worldwide. HFRS is now known to be prevalent in China as well as Korea and pockets of human infection have been found in Japan and in many other countries. New information about the genetic relatedness of virus isolates belonging to the Hantaan group of viruses has been revealed from epidemiologic and molecular genetic studies. Recent epidemiologic studies have reported that a severe form of HFRS has been occurring endemically, and in outbreaks, in the Balkans of Europe. A milder form of the disease has been reported for years in Scandinavia and Eastern Europe. Five distinct Hanta viruses are now recognized which are regionally associated with HFRS: Hantaan virus in Asia; Promala virus in Scandinavia, the western Soviet Union and much of Europe is associated with this less severe form of disease; and Parolya virus found in the Balkans is associated with a very severe form of disease. Another Hanta virus has been isolated from rats in Seoul, Korea. The Seoul virus has the rat as a natural reservoir host whereas Hantaan virus has the *Apodemus* field mouse as a reservoir. A virus very similar to the Seoul virus has been isolated from rats in many countries of the world, especially near shipping ports. Although the Seoul virus is not known to cause disease in man, serologic evidence of human infection has been obtained in Baltimore, Maryland. Like other bunyaviruses the Hanta viruses have a three segment genome. Relatedness of these viruses can now be determined from nucleotide sequences.

Rabies

The human diploid cell subunit rabies virus vaccine (HDRV) has proven highly efficacious in postexposure treatment for rabies. Where available the vaccine treatment also includes simultaneous administration of human rabies immune globulin. Only rarely have there been vaccine failures when both vaccine and immune globulin have been properly administered. And in preexposure immunization, HDRV has been highly successful. Unfortunately, the current cost of the vaccine has precluded its generalized use in humans in less affluent countries than the United States and Europe. Other countries still rely on less expensive vaccines produced from infected animal brains. Therefore, development of an inexpensive subunit rabies vaccine has been pursued using recombinant DNA technology. This has successfully been achieved using vaccinia virus genetically modified to contain the rabies virus gene that codes for the envelope glycoprotein. The vaccine was developed for control of rabies in wildlife with the strategy of administering the vaccine though baits. The strategy has been demonstrated successfully in laboratory animals, but has not yet been applied to field studies. If this vaccine can be proven safe and effective in the field, it will have great potential for control of rabies in India and Southeast Asia where wildlife rabies regularly spills over to humans. An estimated 10,000 persons die from rabies every year in this area of the world.

Rabies human immune globulin is becoming unavailable and risky because of potential contamination with other viral pathogens. Use of equine immune rabies globulin is effective, but it also carries the risk of developing hypersensitivities and serum sickness. Therefore, studies have been pursued to develop human or mouse monoclonal antibodies that can replace human immune globulin. Mouse and human monoclonal antibodies that neutralize the rabies viruses *in vitro* have been produced. These monoclonal antibodies also successfully protected laboratory animals against rabies virus challenges in both pre-exposure and post-exposure situations. Production of monoclonal antibodies is still problematic from human lymphoid tissue, but not so from transformed mouse tissue. Clinical trials are planned for testing the safety of these monoclonal antibodies in humans.

The contribution of lymphoid cells to acquired protection against rabies infection is still little understood. Studies are attempting to determine the relative role of different effector mechanisms induced by immunization with rabies virus immunogens. T cells such as helper T lymphocytes or cytotoxic T cells may both contribute to the prevention of a rabies virus infection and virus spread throughout the nervous system. Rabies virus ribonucleoprotein (RNP) was shown to be a major target antigen for T helper cells that cross-react between rabies and rabies-related viruses. Immunodominant T helper cell epitopes of the viral nucleoprotein were identified *in vitro* by using synthetic peptides delineated from the amino acid sequence of the nucleoprotein. The response to synthetic peptides was under the host Ir gene control. These studies suggest that antibodies directed against RNP are an important component of the protective response against rabies virus infection. The function of this RNP-specific antibody may be the promotion of the attachment of viral antigen to phagocytic cells, which are then stimulated to produce monokines such as interferon that could inhibit viral replication.

SIGNIFICANT ACCOMPLISHMENTS

Summary of the First Five Years—1965–1969

Research was oriented toward viral diseases of Asia including smallpox, rabies, and the arthropodborne viruses.

A gel-precipitation test developed for diagnosis of smallpox is specific, rapid, and is 92% as sensitive as the more time-consuming technique of virus isolation. Analysis of subunit antigens of variola virus showed the presence of three specific hemagglutination antigens and a high molecular weight precipitin which reacts with a neutralizing antibody.

The nucleocapsid of rabies virus is composed of RNA plus a single protein, which fixes complement. The coat glycoprotein is the hemagglutinating component. A newly developed hemagglutination test is comparable in its accuracy to the serum-neutralization test. In pathogenetic studies in foxes, it was shown that rabies virus matures on plasma membranes in the salivary glands. In nerve cells, intracytoplasmic maturation dominates. A search for clarifying overwintering mechanisms of Japanese encephalitis virus in Korea and for attenuated vaccine strains was unsuccessful. Eleven mutants of *Culex tritaeniorhynchus*, the principal vector of Japanese encephalitis, were developed and show promise for control by genetic manipulation of mosquitoes.

Dengue type-2 virus was shown to contain three proteins, a coat glycoprotein of 59 kd, a core protein of 13.5 kd and a capsid protein of 7.7 kd. The core protein was missing in incomplete hemagglutinating particles. This type of information is essential for vaccine development.

Summary of the Second Five Years—1970–1974

During the second 5 years, research emphasis was placed on rabies, dengue and dengue hemorrhagic fever, Japanese encephalitis, and other arboviruses. Much attention was given to the mosquito, *Culex tritaeniorhynchus*.

A new, inactivated rabies virus vaccine (HDRV) was developed in human diploid cells, and new information was gained on immunopathogenic mechanisms as a cause of early deaths in rabbits. The production of rabies vaccine in many countries depends on the use of neural tissues. Hence, the new HDRV vaccine represented an important scientific advance. Other serologically related viruses were isolated in Africa and were identified as members of rabies-virus complex.

The pathogenesis of dengue hemorrhagic fever is improperly understood. In pursuit of a dengue virus vaccine, heat-sensitive mutants of the virus have been developed. Variations in vector competence for dengue type 2 virus were demonstrated in *Aedes albopictus* taken from different geographic areas. Genetically differing strains of *C. tritaeniorhynchus*, a major vector of Japanese encephalitis virus in Asia, have been developed. These studies are aimed at finding viable strains which would be poor vectors. Yet, they might compete genetically with wild populations and replace the wild vector populations with inefficient vectors.

Summary of the Third Five Years—1975–1979

The mechanisms of dengue hemorrhagic fever/shock syndrome remains unclear. Severity of disease is not related to transmission by certain vector species. Immune enhancement of dengue virus infection was well established, which suggests that entry of dengue virus antibody complexes into mononuclear phagocytes occurs via Fc receptors on these cells. Products are then released which alter vascular permeability. Several structural and nonstructural viral proteins were described for St. Louis and dengue viruses. The mechanism of replication of flaviviruses is different from that of alphaviruses. In flaviviruses, ribosomes recognize internal initiation sites on genomic messenger RNA. This is in contrast to alphavirus translation, which begins at terminal sites, with production of precursors that are subsequently cleaved.

Vector competence studies showed the relationship of the midgut barrier to virus infection and dissemination within the mosquito host. Other studies with California group bunyaviruses and their *Aedes* vectors revealed that transovarial transmission of arboviruses occurs commonly. Transovarial transmission of Japanese encephalitis virus and St. Louis encephalitis virus and dengue viruses occur in several species in the laboratory, but their importance in the field has not been established.

Korean hemorrhagic fever (hemorrhagic fever with renal syndrome) is present in the wild rodent *Apodemus agarius*. Cells from these rodents were used to isolate the agent from humans. The disease is now known to occur in Japan, thus stimulating new interest in this disease by the Viral Diseases Panels.

The research goals of the panels were officially expanded in 1978 to include viral gastroenteritis because of the severe problem of these diseases in Asia and other areas of the world, including the United States and Japan. The structure of rotaviruses was demonstrated. Human rotavirus type 2 was successfully propagated in cell cultures. Epidemiological studies have shown rotaviruses, adenovirus and non-cultivatable 27nm viruses responsible for acute viral gastroenteritis in humans. Viral infections were detected by ELISA and immunoelectron microscopy. Rotaviral infections were most common. Rotavirus antibody prevalence was shown to increase with age. Sporadic outbreaks of Norwalk-type (27 nm particles) occurred in the United States.

The human diploid cell inactivated rabies virus vaccine consistently produces antibodies in human recipients. Tests of an oral rabies vaccine for animal administration resulted in antibody production. A guinea pig model system for post-exposure testing of rabies vaccine efficacy was developed. Oligonucleotide fingerprints showed that fixed rabies virus RNA differs from wild strains. A monoclonal antibody system showed that the glycoproteins antigens were diverse. Strains from the same geographic area and species of origin have similar patterns of reactivity. Natural antigenic variation may explain g-failures of post-exposure treatment of rabies. Cell to cell spread of rabies virus occurs by budding of virus from one cell directly into cytoplasm of an adjacent cell.

Summary of the Fourth Five Years—1980–1984

Research goals of the panels were again expanded slightly in 1983 to include hemorrhagic fever with renal syndrome (formerly Korean hemorrhagic fever) as well as viral gastroenteritis, rabies, dengue, and other arboviruses. Several advances in biotechnology including monoclonal antibodies, RNA fingerprinting by two-dimensional radiochromatography and nucleotide sequencing did much to advance our knowledge of virus structure, viral gene functions, replication, and pathogenesis. These technologies also offered new opportunities for vaccine development.

In arbovirus research considerable attention was given to genomic reassortment by bunyaviruses as genetic mapping of this three segmented genome was undertook. Much of the studies involved La Cross virus and related California group viruses because of their ease of culture and assay. It was found that the mid-length RNA segment, M, carried the genetic information for virulence in the mouse and transmision capabilities in the arthropod vector. Eight antigenic sites were found on the G1 protein using monoclonal antibody technology. The small segment, S, codes for nucleoprotein and a nonstructural protein. The L segment codes for viral transcriptase. Segment reassortment allows for genetic heterogeneity. Segment reassortment is restricted to closely related virus isolates or strains. It does occur among viruses of different genera.

Research sponsored by the U.S. panel led to better understanding of Old World and African area viruses. GP-1 and GP-2 glycoproteins of lymphocytic choriomengingitis virus and Lassa and Mozambique viruses were studied. Definitive evidence of the conservation of genetic information coding for GP-2 among these three viruses and their close links was demonstrated.

Studies also focused on alphavirus transcription. Viral minus strand RNA is the template of synthesis for two species of plus strand RNA—the 49S virion RNA and 26S messenger RNA. Minus strand synthesis is required to amplify virion RNA in infected cells. Complementary DNA (cDNA) corresponding to the genes coding viral glycoproteins fo two temperature-sensitive mutants of Sindbis virus were sequenced.

Other studies with dengue virus and aedine mosquitoes other than *Aedes aegypti* showed a New World species, *Aedes (Gymnometopa mediovitatus)*, occupies the same ecologic niche as *A. aegypti* and bites man readily. It is susceptible to oral infection with dengue 1 virus and is more susceptible to dengue 2 virus infection than a highly susceptible strain of *A. aegypti*. Of particular interest is the demonstration of transovarial transmission of dengue virus in *A. mediovitatus*. It showed the highest overall filial infection rates observed for any flavivirus including recorded rates for *A. aegypti* with all four dengue types.

Progress in classification and understanding of rotaviruses and their structure, composition, and function was rapid. Swift progress was largely due to stimulating research reports presented at Joint Panel meetings, constant communication between U.S. and Japanese rotavirologists and active collaboration with exchange of information, reagents, and scientists. The major advances include: a cell culture system for isolating and characterizing viruses and a refinement of the cell culture system by adding trypsin to amplify yields and allow for plaquing of viruses. This double-shelled virus revealed five proteins as components of the inner shell and four proteins in the outer shell. Rotavirus neutralizing antigen contains type-specific and type-common determinants. Both genomic segments 3 and 9 (out of a total of 11) code for type-specific neutralization. A mouse model to study homologous and heterologous immune responses is under development.

The Norwalk agent is a common cause of gastroenteritis outbreaks in the United States; by early childhood, this virus is found throughout the world in high frequency. Studies were initiated to characterize a newly described Snow Mountain agent. It is morphologically similar to Norwalk and Hawaii agents but is antigenically distinct. Antibody to these viruses was found to be acquired late in childhood; prevalence is approximately 60% in selected adult populations throughout the United States.

Considerable progress led to better understanding of the occurrence and viral characteristics of the agents which cause hemorrhagic fever with renal syndrome (Korean hemorrhagic fever). In work reported at Joint Panel meetings, Hantaan virus, the designated etiologic agent, was isolated in cell culture from clinical cases and wild rodents in Korea and Japan. This virus is a bunyavirus with a three-segment, single-stranded, RNA genome. These observations stimulated research elsewhere, and related viruses have been isolated from rodents in the United States and Europe. These findings suggest a close association with rodents and worldwide distribution. Biochemical and antigenic analysis of these isolates indicate that they form a complex designated Hantaan virus group.

The development of human diploid cell inactivated rabies virus vaccines, and more effective killed and live attenuated animal vaccines stimulated interest in evaluation of rabies virus antigens and the relationship of these antigens to human protection. Studies showed the existence of at least three distinct antigenic sites on the rabies virus glycoprotein that is an essential component of effective vaccines. Some strains of fixed virus show four sites. Mutations in the nucleotide sequences were identified in ERA and CVS strains of virus that may account for the nonpathogenic variant of virus.

Summary of the Fifth Five Years—1985–1989

The advent of new biotechnology using recombinant DNA techniques, monoclonal antibodies, and immunologic methodologies has revolutionized many aspects of this program of research and caused an explosion of new information about the viruses, their antigens, and new approaches to vaccine development.

Several candidate vaccines for protection against rotavirus infections have and are being developed. These include live, attenuated reassortant virus vaccines, host restricted live virus vaccines, cold-adapted live virus vaccines, and subunit vaccines produced by recombinant DNA techniques. Major advances were achieved by the development of cell cultures for propagation and assays of rotaviruses, of monoclonal antibodies to rapidly identify serotypes, a plaquing technique for isolation of viruses and neutralization studies and cloning and sequencing of all 11 genome segments for structure/function studies and production of gene products. The most promising of the candidate vaccines to date are the live virus reassortants from rhesus monkey rotavirus and human rotaviruses.

Outstanding progress has also been achieved in arbovirus research. Genomes of several alphaviruses and flaviviruses have now been cloned and sequenced, including Sindbis virus, dengue viruses types 2, 3 and 4, yellow fever virus, Japanese encephalitis virus, St. Louis encephalitis virus, Eastern equine encephalitis, and Western equine encephalitis viruses. WEE virus has been shown to be a natural recombinant of Sindbis virus and EEE. Subunit vaccines for dengue viruses have been produced and tested in mice and monkeys. Although good protection in mice was achieved, protection in monkeys was not as obvious. Major advances have achieved in genetic mapping of arboviruses and identification of protective antigens. The use of nonstructural proteins of the viruses in vaccines may be essential for maximum efficacy.

Four distinct viruses of the Hanta group of bunyaviruses have now been recognized. Three of these viruses cause hemorrhagic fever with renal syndrome. The Hantaan virus is associated with disease in Asia, Parolya virus is associated with very severe disease in the Balkans, and Promala virus is associated with mild disease in Scandinavia, eastern Europe, and the western Soviet Union. A fourth virus, Seoul virus, has been isolated from rats in several countries of the world but is not yet associated with human disease even though antibodies to the virus have been detected in humans in Baltimore, Maryland. Relatedness of these viruses can now be determined from nucleotide sequences.

The human diploid cell rabies virus vaccine (HDRV) has proven highly efficacious in post-exposure treatment for rabies as well for pre-exposure prophylaxis. When available, the post-exposure treatment also includes simultaneous administration of human rabies immunoglobulin. Because the cost of HDRV has precluded widespread use in developing countries where human rabies is a severe problem, other vaccines derived from rabies infected animal brains are still in use despite their risks of neurologic impairment. Therefore, development of a relatively inexpensive subunit rabies vaccine has been developed using recombinant DNA technology. Vaccinia virus has been modified to contain and express the glycoprotein gene of rabies virus. The vaccine is intended for control of wildlife rabies by means of vaccine impregnated baits. Animal protection studies have demonstrated the strategy is highly effective for raccoons, foxes, and dogs in a laboratory setting, but it is still untested in field studies. If this vaccine can be proven safe and effective in field trials it will have great potential for control of rabies in India and Southeast Asia, where wildlife rabies regularly spills over to humans. Rabies immune globulins that are administered with HDRV in post-exposure treatments is in short supply. Alternative methods of producing rabies immune globulins have been developed using monoclonal mouse and human antibodies. Field tests are planned for safety and efficacy.

MAJOR RESEARCH MILESTONES

- 1967 marked the first year of joint working conferences between the U.S. and Japanese Viral Diseases Panels.
- A worldwide program for eradication of smallpox through vaccination and surveillance for new cases was initiated in 1968. The program was declared a success 3 years later, and that success has endured so that vaccination against smallpox is no longer required anywhere in the world. The advent and success of the WHO eradication program allowed for the removal of smallpox from the research goals of the Viral Disease Panels.
- The concept of antigenic drift versus shift to explain antigenic variation in influenza viruses was introduced in 1967. This concept has dominated studies of influenza epidemiology and vaccinology for the last two decades. The initiation of other international programs on influenza was partially responsible for the deletion of influenza research from the U.S.-JCMSP guidelines and goals in 1973.
- The successful development in 1970 of continuous cultures of mosquito cells allowed the initiation of studies of the dynamics and kinetics of arthropod-borne viruses in invertebrate cells. Important comparative virologic data between infections in continuous and primary mammalian and avian cells and in invertebrate cells became technically feasible and actively pursued.
- The development of the human diploid cell line, WI-38, led to the successful culture of rabies virus in a medium that was free of potential allergenic, carcinogenic, and encephalitogenic reactions in humans. By 1974 a new subunit rabies virus vaccine was developed and by 1978 replaced the duck embryo rabies virus vaccine used in the United States for pre- and post-exposure treatment of rabies.
- A new killed rabies virus vaccine prepared from virus grown in chick embryo cells was developed in Japan almost simultaneously with the HDRV vaccine in the United States. The chick embryo rabies vaccine is immunogenic and free of encephalopathy-generating material. It is considerably cheaper to produce than the HDRV vaccine and, thus, has greater potential for use in Asia.
- The etiologic agent of Korean hemorrhagic fever (hemorrhagic fever with renal syndrome) was identified by immunofluorescence in the lungs of the Korean field mouse, *Apodemus agrarius* in 1970. The virus was isolated and characterized over the next several years as a bunyavirus.
- In 1970 the viral etiology of the Norwalk-like outbreaks of nonbacterial gastroenteritis was established by immunoelectronmicroscopy (IEM). A 27nm virus particle was described.
- In 1974 a reolike virus (later classified as rotavirus) was identified by IEM as an etiologic agent of viral gastroenteritis in infants and children. Viral gastroenteritis, because of its devastating morbidity and mortality in infants and young children, was included in the research goals of the USJ-CMSP in 1978.
- Human rotaviruses were successfully cultivated in continuous cultures of monkey cells in 1979 which allowed *in vitro* studies with pure cultures of virus. This advancement quickly led to the characterization of the 11 segment genome and then to segment-reassortment studies. Soon thereafter, a method of plaquing the human viruses was devised by using trypsin to enhance virus-cell adsorbtion. Plaquing allowed for quantitation of infectious virus particles and greatly enhanced the pace of research on these pathogens.
- A live reassortant rotavirus was constructed by dual infection of monkey cells with rhesus monkey rotavirus

and human rotavirus in 1985. This reassortant became the prototype for promising new candidates for human rotavirus vaccines that currently are being evaluated in clinical trials.

- In 1976, the ability of arboviruses to be perpetuated by transovarial transmission in their arthropod host was successfully demonstrated in the laboratory. The initial observations with LaCross virus, a bunyavirus, were later confirmed and extended with alpha-and flaviviruses. This mechanism of persistence helps to explain the overwintering of arboviruses in mosquitoes. Venereal transmission of arboviruses from infected male mosquitoes to females was also demonstrated as a horizontal mode of perpetuation; i.e., infected male larvae upon maturity could pass the virus venereally to uninfected females that feed on mammals.
- The technology of RNA fingerprinting was successfully adapted to arbovirology in 1976. This technique of radiochromatography allowed the comparison of viral genomes and segments of viral genomes. In turn, the technology led to genetic reassortant experimentation and gene function studies at a molecular level. The first application was to bunyaviruses because of their three-segment genome. Subsequently, the techniques was used for genetic epidemiology of dengue viruses, defining geographic strains of dengue virus with greater precision than serologic methods allow.
- In 1984 the genome of Sindbis virus, a prototype alphavirus was successfully sequenced by cloning cDNA genomic segments using plasmid vectors and bacterial hosts. Several other alphavirus genomes were subsequently cloned and sequenced. As the single open-reading-frame for each virus was determined, it became apparent that the entire genome is translated as one protein that is subsequently processed to structural and nonstructural proteins for viral replication and assembly. The new technology led to the discovery that alphaviruses recombine naturally. In 1986 Western equine encephalitis virus was found to be a natural recombinant of Sindbis and Eastern equine encephalitis viruses.
- The genetic code of the 17D strain of yellow fever virus (vaccine strain) was "cracked" in 1985, setting the stage for cloning and sequencing of other flavivirus genomes, including four dengue types. Like the alphaviruses, it was shown that flavivirus genomes are translated into single large proteins that are subsequently processed to structural and nonstructural viral polypeptides. Later, the 17D genome was cloned in two pieces and then annealed to yield infectious RNA. This is considered a major breakthrough to the eventual understanding of the functions of arboviral components and pathogenesis. It is probable that this technological advance will lead to improved vaccines for dengue and other arboviruses.
- Rabies virus genomic RNA was successfully cloned as complementary DNA in 1981. This advancement has led to new subunit vaccines with viral glycoproteins expressed in bacteria, yeast, mosquito cells or mammalian cells, all of which allow for purification ofthe desired immunogens. In 1985 the molecular techniques were extended to modifying vaccinia virus with rabies virus complementary DNA coding for rabies virus envelope glycoproteins for use as a live vaccine in wildlife. This new approach to rabies control has the potential for eliminating the threat of rabies being transmitted from infected wildlife to domestic animals and humans.

PROJECTS SUPPORTED BY THE PROGRAM

Investigator and Institution	*Title*
Margo A. Brinton Georgia State University	Cellular Gene Product Modulates Flavivirus RNA Synthesis
Thomas J. Chambers Washington University	Molecular Biology of Flavivirus Replication
Joseph J. Eiden Johns Hopkins University	Group B Rotaviruses—Structure and Clinical Significance
Mary K. Estes Baylor College of Medicine	Antigenic Variation of Human Rotaviruses
Mary K. Estes Baylor College of Medicine	Subunit Rotavirus Vaccines & Mucosal Immunity
Brian A. Federici University of California Riverside	Biology and Characterization of Ascoviruses
Francisco Gonzales-Scarano University of Pennsylvania	Fusion Function of La Crosse Bunyavirus
Harry B. Greenberg Stanford University	Viral Gastroenteritis: Basis of Virulence and Protection

Ari H. Helenius Yale University	Cell Biology of Virus Entry
John J. Holland University of California San Diego	Synthesis in Normal, Virus Infected and Tumor Cells
Henry V. Huang Washington University	Mechanism of Alphavirus Subgenomic MRNA Synthesis
Robert E. Johnston University of North Carolina Chapel Hill	Molecular Basis of Alphavirus
Hilary Koprowski Wistar Institute of Anatomy and Biology	Study of Rabies and Rabies Virus Antigens
Edward J. O'Rourke Children's Hospital (Boston)	Biology and Immunopathology of Dengue Virus Infections
John T. Patton University of Miami School of Medicine	Viral Proteins in Rotavirus Replication
Robert F. Ramig Baylor College of Medicine	Genetics of the Rotaviruses
Patricia M. Repik Medical College of Pennsylvania	Molecular Investigation of the Ecology of EEE Virus
Charles M. Rice Washington University	Molecular Genetics of Sindbis Virus Replication
Leon Rosen University of Hawaii at Manoa	Transovarial Transmission of Dengue Viruses
Dorothea L. Sawicki Medical College of Ohio at Toledo	Regulation of Alphavirus Transcription
Sondra Schlesinger Washington University	Structure and Replication of Enveloped RNA Viruses
Thomas W. Scott University of Maryland College Park	Togavirus—Vector Interactions
Thomas W. Scott University of Maryland College Park	Rapid Transmission of EEE Virus by Culiseta Melanura
Victor Stollar R. W. Johnson Medical School	Arbovirus Replication in Mosquito and Vertebrate Cells
James H. Strauss California Institute of Technology	A Genetic Analysis of Sindbis Virus
James H. Strauss California Institute of Technology	Togavirus Replication Strategies and Evolution
Robert B. Tesh Yale University	Support for World Reference Center for Arboviruses
Gregory H. Tignor Yale University	Rabies Serogroup Virus Infections: Immunopathology

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Japanese Viral Diseases Panel

BROAD GOALS

The goals and objectives originally (1965) identified for the Viral Diseases Panels were broad and included diseases caused by arboviruses, respiratory diseases caused by influenza, parainfluenza, respiratory syncytial and adenoviruses, smallpox, and rabies. At the Ninth Viral Diseases Panel Meeting in 1975, it was mutually agreed that research areas would be focused on rabies, dengue, and other arboviruses in accordance with the recommendations made by the Joint Subcommittee and Committee of the Program. In 1978, at the 12th Joint Viral Diseases Panel Meeting, the Panels's guidelines were extended to include viral gastroenteritis and hemorrhagic fever with renal syndrome (HFRS) also was incorporated.

At present, the scope of the research areas of interest to the Panels includes important and prevalent diseases common to the United States, Japan, and Asia. These are dengue, dengue hemorrhagic fever, other arthropodborne virus diseases, HFRS, rabies, and viral gastroenteritis, all of which pose important problems as threats to human health in many parts of the world. The Program is collaborating with common research guidelines between the U.S. and Japanese Panels. These guidelines include epidemiology, pathogenesis, molecular biology, diagnosis, prevention, and control measures for rabies, arbovirus infections, HFRS, and viral gastroenteritis.

The Joint Panel activities are characterized by the close collaboration and continual communication between U.S. and Japanese scientists. The Joint Panel Meetings will continue to provide a forum for problem identification and resolution in the focused research areas.

PROGRESS IN PRIORITY AREAS

Viral Gastroenteritis

During the last 5 years, continuous progress has been made in many aspects of rotavirus and other diarrhea viruses, and their infections, including molecular biology, cell-virus interaction, epidemiology, immunology, and vaccine development. Such progress was largely due to constant collaboration and cooperation between U.S. and Japanese scientists with the exchange of information, reagents, and personnel.

Rotaviruses have been classified into several groups (A–F) according to their respective antigen specificities. Among them, group A rotaviruses are most commonly detected and often associated with diarrheal diseases in infants and young children throughout the world. At least four serotypes among the group A human rotaviruses (HRV) have been recognized by neutralization tests and serotype specificities are attributed to an outer capsid glycoprotein, VP7, although VP4, another outer capsid protein also elicits neutralizing antibody. Monoclonal antibodies (MAs) directed at VP7 and specific for each serotype have been produced and applied to an enzyme-linked immunosorbent assay (ELISA) for serotyping HRV isolates. The ELISA was utilized for studies on serotype prevalence in epidemics at various locations in Japan. Dominant serotypes were observed in each epidemic; they were distinct not only in different locations at the same time, but also at different times in the same locations. In addition to these regional and temporal variations of HRV serotypes prevailing, HRV strains with unusual antigenic characteristics and unique RNA patterns were detected. The occurrence of these unusual strains as well as regional and sequential variations of serotype dominance may provide important insights into changes by reassortment of genes and the appearance of new, stable mutants; the etiologic and epidemiologic significance of such mutants remains to be determined.

In terms of protection against HRV, the possibility of heterotypic immune resistance was examined in infants who were exposed to recurrent outbreaks of HRV infections in an orphanage. It was shown that pre-existing neutralizing antibodies, though protective against homotypic HRV infection, were not capable of cross protection against heterotypic HRV infections. On the other hand, the presence of cross-reactive neutralizing epitopes on VP4 of HRV were shown by using neutralizing (N) mAbs with a variety of interserotypic reactivities. Serological analysis with six cross-reactive N-mAbs demonstrated antigenic variations in HRV strains with the same serotype, showing the existence of at least three antigenic epitopes on VP4. Collaborative studies between Japanese and U.S. researchers demonstrated that the amino acid substitution on VP4 of mutants selected under the individual N-mAbs were at positions critical to the epitopes for neutralization; a synthetic peptide including the epitope reacted with its corresponding N-mAb. These experimental data may offer an important basis for the development of a vaccine against HRV.

VP4 is associated with biological functions involved in virus-cell interaction such as hemagglutination, growth restriction in cell culture, and virulence. The treatment of HRV with trypsin cleaves VP4 and enhances its infectivity with a concomitant loss of the hemagglutinin activity. Trypsin-treated, infectious HRV was shown by electron microscopy to enter the cell by direct penetration through the cell membrane, whereas untreated HRV has taken up into cells by the process of endocytosis. These morphologic findings were confirmed by biochemical studies. VP7 is the HRV cell attachment protein and adsorption of HRV via VP7 is independent of trypsin treatment. The limited cleavage of VP4 by trypsin is essential for the development of HRV infection and is necessary for the selective internalization/penetration of the inner capsid components, a process that is essential for HRV infection. However, the molecular mechanism by which the trypsin-treated, infectious virions are internalized via VP4 remains unresolved; VP5, the larger cleavage product of VP4, has been shown to possess the neutralization region similar to the putative fusion sequences of Sindbis and Semliki forest viruses.

Several groups in the U.S. and Europe have been developing oral rotavirus vaccines for human use. They are mostly attenuated animal rotaviruses or reassortants. A trial of the rhesus rotavirus vaccine, MMC-18006, was carried out in Japanese infants. All the vaccinees given the vaccine two times, 3 or 6 months apart, showed high levels of neutralizing antibodies against the vaccine strains of HRV. However, they showed only insufficient antibody responses to other serotypes of HRV after booster inoculation of the vaccine. The results appear to agree with the evidence observed in natural infections.

On the other hand, a cold-adapted HRV strain, IGV-80-3 was established as a candidate for human vaccination in Japan, and its immunogenicity was demonstrated in mice and piglets.

Besides typical group A rotaviruses, atypical rotaviruses have been discovered among humans as well as animals. Group B HRV has been prevalent in China causing extensive outbreaks of acute gastroenteritis. On the other hand, group C rotavirus was found frequently in Japanese children with acute diarrhea in the Matsuyama district, and serologically suggested to have been prevalent there. Although these atypical HRV's have not been widespread outside of China or Japan so far, adequate understanding of epidemiology of the virus has not yet been achieved.

Small, round viruses including Norwalk virus are major causes of nonbacterial, epidemic acute gastroenteritis that occur in communities or institutions affecting adults and older children. None of these viruses, however, can be grown in tissue culture. A specific diagnosis of infection with any agent still remains essentially based on electron microscopy, although serologic tests for the diagnosis of Norwalk virus and calicivirus have been developed. As a novel diagnostic technique, a Western blot assay system using SRV-specific antigens in stool extracts was developed for serodiagnosis.

Arboviruses

Substantial studies have been conducted in epidemiology, ecology, laboratory diagnosis, and molecular biology of arboviruses, especially on Japanese encephalitis (JE) and dengue viruses; causative agents of human diseases of public health importance. Rapid advances in molecular biology in recent years have enabled one to analyze genome structure of these viruses, and the results have been utilized to understand their biological characteristics, such as virulence and strain differences, epidemiology and ecology at a molecular level. The results have also been used to develop the second generation vaccines while the current formalin-inactivated and highly purified JE vaccine derived from infected mouse brains has been reevaluated for its efficacy by the laboratory and field tests.

In Japan, the annual number of human JE cases significantly decreased after the epidemic in 1966, and has remained under 100 since 1972. However, the virus persists in nature and its overwintering still is an unsolved question. Oligonucleotide fingerprint analysis on JE virus isolates showed that Japanese strains were generally similar and were significantly different from those in Southeast Asia. Strain differences of JE virus isolates have been analyzed also by a battery of monoclonal antibodies (MCA). Epidemiological studies in Hokkaido, the northern island of Japan which was regarded as a JE- nonendemic area, isolated JE virus from aborted swine fetuses from mothers which could have been infected during the winter months, and the isolated virus showed different MCA-reactivity from those on the main Island of Japan. The study also showed that *Culex tritaeniorhynchus*, the main vector of JE in main and southern islands of Japan, was not a predominant species captured during epidemic season. The data indicate overwintering of JE virus in respective local areas, although vectors and the transmission mode in Hokkaido, especially in the winter season, have not yet been clarified. The marked decrease of human JE cases in Japan has been attributed to the reduced vector population and mass-immunization with the current vaccine. The reduction of the vector has been regarded as the result of wide-spraying of pesticides in its breeding sites of watered rice fields. However, the vector captured in the field in Japan during recent years showed significantly elevated resistance to organophosphorus insecticides compared with laboratory-maintained strains colonized before the wide-spraying of pesticides. Field collection of the vector in Toyama Prefecture in Japan showed its increasing trends in recent years, alarming workers that JE has not been eradicated from Japan.

Several modifications of enzyme-linked immunosorbent assay (ELISA) have been devised for the laboratory diagnosis and serological surveillance on JE, dengue and related flaviviruses.

Field studies and observations in Thailand and Vietnam showed that both JE and dengue virus infections have been serious public health problems in these countries, and development of inexpensive, safe and effective vaccines as well as their rapid laboratory diagnosis were highly required.

Molecular Biology and Development of the Second Generation Vaccines

Since genone sequences of yellow fever (YF) and West Nile (WN) viruses were published in 1985, many other flaviviruses have been partially or completely sequenced. The 3'-end sequence of JE virus RNA was reported to form a secondary structure similar to YF and WN viruses and entire genome sequences were analyzed for JE and type 3 dengue viruses, showing that they have similar genome organization as other flaviviruses: 5'-noncoding region, followed by a single long open reading frame coding C-PrM-M-E-NS1-NS2a-NS2b-NS3-NS4a-NS4b-NS5 proteins, and 3'-noncoding region, although the numbers of nucleotide (nt) and amino acid (AA) residues in each genome segment were slightly different. These data and cDNA clones have been utilized to develop the second generation vaccines. Expression of JE virus envelope glycoprotein (E) gene by recombinant yeast or *Bombix mori* baculovirus turned out to be of limited success. The gene product was relatively small in quantity and appeared to be degraded by endogenous protease in recombinant yeast, resulting in significantly lower immunogenicity to elicit neutralizing (N) antibodies compared with the current vaccine. Most of the E protein epitopes appeared to be conformational depending on disulfide bonds, which were conserved among flaviviruses and present in the N-terminal side of the E protein, and could be destroyed by various steps to purify expressed E gene products. JE virus E protein epitopes have been analyzed by MCA, and passive immunization with N-MCA protected mice from lethal challenge. Analysis on N-MCA-escaped mutants of JE virus indicated that the authentic N-epitope(s) could be composed by combination of different parts of E protein primary structure. On the other hand, analysis on cyanogen bromide-cleaved fragments indicated that an 8Kd fragment near the C-terminal of JE virus E protein could possess denaturation-resistant N-epitope(s). In recombinant vaccinia and baculoviruses, presence and expression of upstream gene, PrM and/or M, beside E protein gene were crucial to obtain E protein gene products with its authentic antigenicity, especially N-epitopes, and their transport to the cell surface.

Immunogenicity of vaccines, and analysis by MCA showed that JE virus strains could be classified into Nakayama, Beijing, and other subtypes. Comparative sequence analysis on E protein genes of several JE virus strains showed that the gene was conserved as a whole with 1,500 nts which determine 500 AA residues, and that a particular AA substitution from serine to arginine at AA number 417 correlated with subtype determination as Nakayama or Beijing.

In dengue type 4 virus-infected cells, accumulation of core protein (C) was observed in the nuclei by anti-C protein MCA and anti-C protein serum raised against the C-protein expressed by recombinant baculovirus. The biological significance of these findings in relation to virus replication or regulation on host cell metabolism remains to be clarified.

In vitro viral RNA polymerase activity was demonstrated in the cytoplasmic extract of JE or dengue type 1 virus-infected cells. The enzyme activity increased in parallel with the production of infective virus, NS3 and NS5 proteins. The NS5 protein has been regarded as a candidate of viral RNA replicase because of its consensus AA sequence with several other viral RNA replicases.

Although Nakayama strain has long been used for JE vaccine production in Japan and its mass-vaccination was reported to have markedly reduced human JE cases in the Republic of Korea, its efficacy to protect for various JE virus strains was reevaluated, because of the strain variations and wide geographical areas of JE epidemics. Among several vaccines produced with different JE virus strains, Beijing-1 vaccine was shown to protect wider range of JE virus isolates, both by mouse model system and human immunization, providing the scientific support to change vaccine strain from Nakayama to Beijing-1 by the National Institute for Health of Japan.

Summarizing the results, basic studies on arboviruses have been carried out paying close attention to their practical applicability to the epidemiological surveillance, prevention, control or laboratory diagnosis, and to the understanding of biological phenomena at molecular levels.

Hemorrhagic Fever With Renal Syndrome

Hemorrhagic fever with renal syndrome (HFRS) is a serious viral zoonosis spreading throughout in Northern Eurasia. In China and Korea, HFRS was severe and often fatal, and the virus linked mostly to field vole, *Apodemus agrarius*. Recently, urban rats have been found to carry the virus with a mild form of HFRS and it is now recognized as an urban problem in the world.

In Japan, the studies of HFRS have made progress rapidly since 1982, when the causative virus was isolated from a carrier laboratory rat through cell culture. Then, various sero-diagnostic methods were developed for detection of HFRS virus infection in humans and rodents, so that HFRS virus was eradicated from laboratory rats. However, incidence of the infection has been shown in urban rats, *Rattus norvegicus*, living at certain port areas and in scattered rural areas.

During the past 5 years, three main research groups, NIH in Tokyo, Faculty of Veterinary Medicine of Hokkaido University and Institute of Microbial Disease (Biken) of Osaka University were engaged in the characterization of HFRS virus and epidemiological studies of HFRS virus infection in rats.

The NIH group performed sero-epidemiological survey in free living *R. norvegicus* for more than 3 years near Tokyo Bay. Rats less than 3 months old usually showed a lower titer of antibody representing maternal antibody. The positive rate increased concomitantly with the increase in the rat's age.

The Hokkaido group performed an epidemiological survey in an endemic focus of urban rats inhabiting a small dumping ground since 1983. The viruses were frequently isolated from the antibody positive adult rats. The antibody of newborn rats was confirmed experimentally to be transferred from immune dams to their offspring prenatally as well as postnatally. The suckling rats from immune dams, at least up to 8 weeks of age, were protected from infection and death by maternal antibody. In the rat born strains, antigenicity was almost similar among them by cross neutralization test, but virulence to rats was heterogeneous.

The Biken group reported that the hantavirus was isolated consistently from various organs of BALB/C mice. The rate of infected monocytes was found approximately to be 0.1% at three months, and decreased to 0.001% one year after infection. Their results also suggest that T cells play an important role in clearance of virus *in vivo*. Virus-specific cytotoxic T lymphocytes were generated when spleen cells from the infected mice were stimulated *in vitro* with infected syngeneic macrophage.

Rabies

As a model system for the study of peripheral rabies virus infection, adult rats were inoculated with CVS strain of rabies virus directly into the peripheral nerve (mental nerve), showing mimicry to the encephalitic form of natural rabies in man. Numerous minute masses of nucleocapsid (NC) antigens were found in and around normal-appearing neurons in the basal ganglia, cerebellum, hippocampus and neocortex. On the other hand, G antigens could hardly be detected in the CNS at the late stage of infection although both G and NC antigens were clearly demonstrable in the trigeminal ganglia at the early phase of infection.

To analyze the viral antigens on the targeted cells which are recognized by cytotoxic T lymphocytes (CTL) ten murine CTL clones (Thy1 + Lyt1-, Lty2 +) were isolated from A/J mice immunized with not only four rabies virus strains, but also with Duvenhage virus.

All the isolated CTL clones were shown to recognize rabies virus G protein antigen by an assay system using purified G protein incorporated liposomes. The CTL clones were successfully transferred to protect mice infected with rabies virus ERA strain. These results suggest that G-specific CTLs are involved in defense mechanism in mice. In addition, the combined effect of anti-rabies antibodies and the CTLs were observed.

Suppression of the CTL response was confirmed in mice which were intracerebrally (ic) infected with street rabies virus (but not with fixed rabies virus strains). This suppressive activity was abrogated by the prior treatment of the cells with anti-Lyt-2 antibody and complement. Suppressor T cells generated are supposed to inhibit the differentiation of precursor of the CTL in the street rabies virus-infected mice.

In contrast to slow progressing CPE caused by rabies virus at the ordinary temperature (36C), the CPE was greatly accelerated when the incubation temperature was elevated to (39.5–40C) after an initial incubation at 36 C. The earliest change observed in the elevated temperature culture was in lysosomal function. The rapid decrease in the uptake of neutral red dye molecule into lysosomes of infected cells was due to the increase in lysosomal pH. It remained unsolved how the pH change in the lysosomes of infected cells is related to the acceleration of CPE. In addition, most of the viral protein became to the resistant to extraction with the Triton (high salt-containing buffer) after raising the incubation temperature, intimately associated with cytoskeletal vimentin-containing structure.

cDNA clones for each, except L, viral mRNA of rabies virus (HEP strain) were prepared and their nucleotide sequence determined. As expected for the attenuated HEP strain of rabies virus, the amino acid at the position 333 of G protein was glutamine, this finding being consistent with other reported observations that the amino acid at the position 333 of avirulent mutants of ERA and CBS strains was substituted by glutamine, while that of their virulent revertants and virulent parent viruses was arginine. Cloned cDNAs of N and M2 protein gene of CVS strain were also cloned.

Nucleotide sequencing of rabies virus genome and mRNA's revealed some difference in the structure of G gene between the HEP strain and other neurovirulent (ERA and Pasteur) strains: although the size of coding region of G-mRNA (1,575 necleotides long) was the same as that of other strains, total length of HEP G cDNA was 2,059 nucleotides long, being longer than other strains by about 400 nucleotides. This additional sequence was located at 3'-noncoding region. While in HEP virus infected cells, only single type G mRNA (2.2 kb long) was produced, ERA virus produced two species of G mRNA, each in an equal amount (long type, 2.3 kb and a short one 1.9 kb long, respectively), maybe by terminating the transcription at either of two possible terminating sites of the viral genome. They also showed that the short type G mRNA of ERA virus lacked the sequence corresponding to the long 3'-noncoding region of HEP G mRNA.

The expression of rabies HEP G protein gene in BHK cell was performed by using an eukaryotic expression vector pZIP (Neo)SVX(1). Although the distribution of the G protein in the G-cDNA-transfected BHK cells was almost the same as in the virus-infected cells, their glycosylation pattern was somewhat different from that of the virus infected cells. G proteins produced in the G-gene-transfected cells were much more glycosylated and sialated.

Recent studies on the efficacy of rabies vaccine used for postexposure vaccination showed that the prophylactic effect of rabies vaccine was closely related to its interferon-inducing activity *in vivo*. Two groups of adult mice were inoculated with a suckling mouse brain vaccine which induced no detectable interferon in mice, and half of them were also inoculated twice with ten units of interferon. The increase of serum anti-rabies IgM and IgG titer was observed earlier in mice which received interferon as well as vaccine than in those which received vaccine alone. The earlier the time of interferon treatment was, the more significant enhancing effect on the anti-rabies antibody production was observed. These enhancing effects were abolished by pretreatment with anti-interferon antibodies. These results suggest that interferon induced by rabies vaccine might play a role in prophylaxis of rabies by enhancing the antibody production in addition to the direct antiviral activity.

Current Japanese and American tissue culture vaccines are produced by propagating rabies virus in chick embryo cell (CEC) and in human diploid cell (HDC) cultures, respectively. Neutralizing antibody responses were compared between vaccinees who received the Japanese commercial rabies CEC vaccine and those who received the American commercial rabies HDC vaccines by the rapid fluorescent focus inhibition test. Despite lower total protein content, persistent neutralizing antibody titer exceeding 1:40 remained in the vaccinees who received that CEC rabies vaccine for about 1 year after the last shot, as well as in those inoculated with HDC vaccine.

The use of recombinant DNA technology has been applied to rabies vaccine development. The rabies virus G-cDNA was expressed in yeast cells by using the expression vector system. At present the proteins produced are resistant to usual extraction procedures.

SIGNIFICANT ACCOMPLISHMENTS

Summary of the First Five Years

Japanese delegates first met with U.S. Viral Panel members at the working conference on smallpox held in Honolulu in December 1967. Working Conferences on myxovirus infections (1968), immunization against Japanese encephalitis (1969), and rabies (1970) were held alternately in Tokyo and Washington in subsequent years.

Summary of the Second Five Years

In 1973, the joint efforts between the U.S. and Japanese Panels focused on the three major areas of study: rabies, arboviral diseases, and dengue hemorrhagic fever. These included epidemiology and ecology, molecular biology, pathogenesis, preventive measures including environment control and modification of vector competence of mosquitoes, and the development of new vaccines.

Summary of the Third Five Years

Substantial progress was made in the targeted ares. Appropriate laboratory techniques and diagnostic methods developed in the Program were used successfully to expand epidemiological and ecological studies. Studies on molecular biology of the virions were also accelerated.

The epidemiological profiles of viral gastroenteritis in Japan were found to be similar to those in the United States and other developed countries. A major scientific breakthrough in *in vitro* cultivation of human rotaviruses was reported at the 1980 working conference. The field of rabies research was directed toward development of new prophylactic vaccines. Studies of comparative epidemiology and ecology and of pathogenesis of arbovirus infections in the United States, Japan, and other Asian countries made it possible to better understand the diseases and to develop measures for their prevention and control. Spread of Hantaan virus, the etiologic agent of hemorrhagic fever with renal syndrome (HFRS), and its related viruses in many countries was made clear by the international collaboration between researchers in the United States, Korea, and Japan.

The Program guidelines are closely related to the important health problems in Asian countries. For this reason, the Panel has encouraged scientists of these countries to participate in the working conferences.

Summary of the Fourth Five Years

A variety of research was conducted in the field of arboviruses, making considerable progress in the understanding of their epidemiology, immunology, and molecular biology. Japanese encephalitis (JE) epidemics in Nagasaki were demonstrated to be correlated with weather factors in summer, which were related to vector mosquito populations and virus multiplication in the vector. Diagnostic procedures such as biotin-avidin ELISA or IgM capture ELISA were shown to be useful for antibody detection and differential diagnosis between JE and dengue. In JE-virus infected mice, protectivity was noted with B cells but scarce with T cells. Immune B cells seemed to play an important role in cellular immunity with the cooperation of specific antibodies in JE virus infection. Attempts were made to determine the structure of the JE virus genome. Clones over 7 kilobases which occupied more than half of the genome were obtained and sequenced.

The spread of rabies virus in the brain was studied in patients who died of rabies. Viral budding processes were found at the neuronal synapses. The essential role of cell-mediated immunity in the treatment with post-exposure vaccination was demonstrated in mice and in cell culture systems. In HEP rabies virus infected BHK cells, temperature increase induced diverse molecular changes such as the termination of genomic RNA synthesis and marked increase of viral mRNA synthesis without increase of viral protein synthesis. Biochemical analysis of rabies virus proteins revealed that p40 was modified, re-phosphorylated M1 protein and detected only in the mature virion.

A major advance in the field of viral gastroenteritis was a success in *in vitro* cultivation of human rotavirus (HRV), which allowed almost routine isolation of HRV. The successful propagation of HRV facilitated their serotype classification according to neutralization specificities (serotypes 1-4). As a potential live vaccine candidate, cold-adapted HRV strains were developed. Besides HRV, enteric adenovirus (serotypes 40 and 41), calicivirus, astrovirus, and Otofuk agent were detected as causative agents of acute gastroenteritis.

Hemorrhagic fever with renal syndrome (HFRS) agents, "Hantaan virus," were classified as the Bunyaviridae. Antibody prevalence among wild rodents was found worldwide. Much of this progress could be attributed to the close collaboration between U.S. and Japanese scientists established throughout the Program. Several types of Hantaan-related viruses were isolated in Japan.

MAJOR ACHIEVEMENTS

Development of New Rabies Vaccine

A new type of chick-embryo cell-killed rabies vaccine was developed and tested for pre-exposure and post-exposure efficacy.

In order to obtain rabies vaccine free of brain material, the high-egg-passage (HEP)-Fleury strain of fixed rabies virus, cultured in chick embryo (CE) cells, (CEF strain) was adapted to CE cells maintained in serum free medium. Rabies vaccine for human use was made with the CEF virus, inactivated by beta-propiolactone.

Five guinea pigs received two doses of one ml each, weekly, of either CEC vaccine or Semple type, SMB vaccine intraperitoneally. The neutralizing antibody titers obtained with the TC vaccine were slightly higher than those with the SMB vaccine.

In human inoculation tests, seven volunteers were vaccinated subcutaneously with one ml of the unconcentrated TC vaccine and in a week received the same dose. Clinical observation revealed no general reactions but very weak local reactions.

The NT antibody response is not available but HI titers were 1:40 in two persons, 1:80 in one, 1:320 in three, and 1:640 in one at 2 weeks after the first vaccination.

A second study demonstrated the antibody response in volunteers inoculated with concentrated CEC rabies vaccine and postexposure treatment for rabies in humans and primates.

The volunteers received 2 ml of CEC vaccine subcutaneously five times every other day. Good antibody formation (neutralizing antibody) developed in serum specimens. The only side reaction was noted at injection sites about 7 days after the first vaccination.

Five other volunteers evaluated in Bangkok, Thailand, were bitten by dogs known to have rabies, or strongly suspected of it. They received post-exposure treatment with CEC vaccine. Vaccinees produced high level of antibody (neutralizing antibody) and none showed signs of rabies up to 3 months.

Trials of pre- and post-exposure treatment of rabies infection with CEC vaccine continue and are being expanded carefully in Japan and Thailand.

Establishment of *Aedes albopictus*, Clone C6/36, Cell Line and Its Application to Arbovirus Studies

Arboviruses are maintained in nature primarily by biological transmission between susceptible vertebrates by hematophagous arthropods, such as mosquitoes and ticks. They include many human pathogens, especially flaviviruses such as yellow fever (YF), Japanese encephalitis (JE), and dengue viruses. In contrast to vertebrate cell cultures, arthropod cell cultures were infrequently used in animal virology. Epidemiologic and laboratory evaluation of dengue viruses faced the difficulty of *in vitro* virus growth in suitable host cells.

In order to overcome this difficulty, a clone C6/36 cell line was obtained from Singh's *Ae albopictus* cell culture. It was established from a mixture of about 100 larvae, using Mitsuhashi-Maramorosch's medium, containing lactalbumin hydrolysate and yeast extract of unknown composition supplemented with 20% fetal calf serum (FCS). The cell line was first adapted to Eagle's medium containing a known composition of nutments supplemented with 10% FCS and 0.2 mM each of nonessential amino acids. The cell line was then cloned in the presence of anti-chikungunya and anti-dengue serum, in order to remove possible contamination of the cell line by these viruses. The virus yields from each clone were monitored. The finally established clone, C6/36, possessed uniform susceptibility and produced supported higher virus yields compared with the uncloned cell line of mixed population.

Comparative sensitivity tests of the clone C6/36 with classical mouse-brain inoculation used to isolate JE virus from field-collected *Culex tritaeniorhynchus* and human specimens were performed in Japan. These showed similar or better sensitivity of the C6/36 cell line, useful for isolation of several unknown possible insect viruses of mosquitoes and mutants or variants of JE and Getah viruses, not ordinarily detected by the classical method. Virus isolations by the C6/36 cell line was performed in Southeast Asia. Dengue viruses were isolated not only from severe dengue hemorrhagic fever patients but also from persons with mild fever of unknown origin (FUO) as well as cases with encephalitis and meningitis. The results suggested that FUO cases could act as amplifiers of dengue viruses and that some cases with neurological manifestations were caused by dengue viruses, so-called dengue encephalopathy.

In laboratory studies, the C6/36 cell line was grown in mass-cultures in suspension in order to obtain sufficient amounts of purified JE and dengue viruses useful for further analyses. Molecular and epidemiologic studies of JE virus isolates showed that Japanese strains were generally similar but significantly different from those in Southeast Asia. This indicated that virus mutation and selection had proceeded independently in geographically distant areas. Molecular cloning and sequencing studies of JE and type 3 dengue virus genomic RNAs clarified their entire nucleotide sequences. This revealed their similar genome organization to other flaviviruses, such as YF and West Nile viruses. This new data and cDNA clones have been helpful in developing recombinant vaccines, and better clarification of the biological characteristics of these viruses at a molecular level.

Propagation of Human Rotavirus in Cell Culture

A major contribution of Japanese rotavirologists was establishment of a cultivation system of human rotavirus (HRV). HRV strains were successfully propagated from fecal specimens in cell culture using a combination of techniques. These included roller cultures of MA104 cells, pretreatment of specimens with trypsin, and addition of low levels of trypsin to the maintenance medium. Subsequently, a number of HRV strains were propagated using similar methods. It was found that primary cynomolgus monkey kidney cells were more sensitive than MA104 cells.

In other studies, fecal specimens were obtained from patients with whitish, watery diarrhea, vomiting and slight fever, which were shown by electronmicroscopy to contain rotavirus particles. Cultures of MA104 cells were then prepared in 110 X 10 mm tubes. A 20% fecal suspension in PBS was centrifuged and the supernatant fluid was treated with 10 ug/ml trypsin (Sigma) at 37°C for 30 minutes. Serial dilutions of the treated material were inoculated in 0.1 ml volumes into cultures of MA104 cells. After incubation at 37°C for 60 minutes the cultures were washed three times, supplemented with 0.5 ml of the maintenance medium containing 1.0 ug/ml trypsin and incubated in a rolled drum at 37°C. Further passages were made at intervals of 3 days. The culture fluids for passage was treated with 10 ug/ml trypsin.

Cytopathic effect was not observed in any of the primary cultures. On the second passage, cytopathic effect was observed, and during the third passage it was obvious; the infectious titer of the fluid phase was $10^{4.3}$TCID 50/0.1 ml. Immunofluorescence was positive up to a 10^{-5} dilution.

The strains isolated were designated as "Hochi", "Ito", and "Toi". Results of neutralization tests indicate that the new isolates are closely antigenically related with strain Wa.

FUTURE GOALS

Gastroenteritis

Rotavirus is a major cause of severe diarrhea in infants and young children, and its high morbidity and mortality in the developing world warrant active investigations aimed at development of vaccines. Further studies are needed on molecular characteristics, pathogenesis, and immunology of human rotavirus. In addition to group A rotavirus, atypical rotaviruses with other group specificities have been thoroughly investigated in regard to their incidence, epidemiology, and viral characteristics. Additional work on characterization of small, round viruses, including Norwalk agent, and their etiological significance is also needed. Further understanding of the epidemiology of viral gastroenteritis in the developing world is required in terms of the diversity of etiology and their relevant serologic responses. The development of effective preventive and control measures for the illness is a long term goal.

Arboviruses

Since the U.S.-Japan Cooperative Medical Science Program was started to control major diseases in Asia, especially in Southeast Asia, future studies in the field of arboviruses should be directed toward diagnosis, control, prevention, and treatment of Japanese encephalitis (JE) and dengue hemorrhagic fever (DHF). The first generation JE vaccines have been developed and successfully used to control JE in Japan and the Republic of Korea. The amount of available vaccine is still not enough and cost is still high for mass-immunization in several other countries and dengue vaccines are still under development. Since genetic structures of these viruses have already been clarified, the results should be applied to develop the second generation JE and dengue vaccines. This work has already been started by the expression of their protective epitopes by recombinant DNA technologies, however, further studies will be required for their practical application. The expressed gene products will be used as reagents in specific and sensitive immunological tests, and cDNA probes and monoclonal antibodies will be applied to the rapid diagnosis and epidemiological surveillance. This information will be practically important for the proper treatment and control programs. Molecular studies on the replication and virulence of these viruses will provide useful information for the development of antiviral reagents and genetically engineered live attenuated vaccines.

Hemorrhagic Fever With Renal Syndrome (HFRS)

Main research on HFRS in Japan will be directed to both ecology of hantavirus and HFRS vaccine development.

Field surveys of hantavirus infection among urban rats in the endemic areas must be continued further to elucidate the natural transmission of the virus. Laboratory experiments using bloodsucking gamsad mites are designated to determine whether hantavirus is transmitted by the mites, or not. The mechanism of persistent infection in the presence of antibody *in vivo* would provide more basic information on the roles of both humoral and cellular immunity. The comparative molecular genetics among hantaviruses would give more exact results on the analysis of virulence, the determining of evolutional background and the development of a recombinant vaccine.

PROJECTS SUPPORTED BY THE PROGRAM

Investigator and Institution	*Title*
Jiro Arikawa Hokkaido University	Studies on neutralizing determinants of the envelope glycoprotein hantavirus
Shunzo Chiba Sapporo Medical College	Studies on immunological responses of rhesus rotavirus vaccine MMU-18006
Kenji Fukudome Tohoku University	Effects of glycoproteins and lectins on hemagglutination and infection of human rotavirus
Norio Fukuhara Tohoku University	Studies on entry of human rotavirus into cells
Nobuo Hashimoto Hokkaido University	Surveys of hantavirus infection among urban rats in Japan
Akira Igarashi Nagasaki University	Japanese encephalitis and dengue hemorrhagic fever in Vietnam
Nobuko Ikegami Osaka National Hospital	Serotypic analysis of endemic prevalence of group A human rotavirus
Yuzo Iwasaki Tohoku University	Experimental rabies in the rat
Akihiko Kawai Kyoto University	Genome structure of rabies viruses Effect of temperature up-shift on rabies virus infection in culture
Setsuko Kitaoka Tohoku University	Serological characteristics of a unique human rotavirus strain AU-1
Nobumichi Kobayashi Sapporo Medical College	Studies on unusual human rotavirus strains
Tasuke Konno Tohoku University	Anti-idiotypic antibodies of human rotavirus VP3 antigen
Takeshi Kurata National Institute of Health Tokyo	Comparative pathology of hemorrhagic fever with renal syndrome in human and animals
Kazuaki Mannen Medical College of Oita	Nucleotide sequence of rabies virus cDNA for the N and M proteins
Shigeo Matsuno National Institute of Health Tokyo	Studies on cold adapted human rotavirus

Kumato Mifune Medical College of Oita	Immunopathology of rabies infection
Kenjiro Morimoto Kyoto University	Biochemical and immunological studies on the Gs protein of rabies virus
Osamu Nakagomi Akita University	Molecular characterization of human rotavirus strains
Akira Oya National Institute of Health	Evaluation of immunization of pigs for the control of Japanese encephalitis epidemic
Hideo Sumiyoshi Tokyo Institute of Medical Science	Nucleotide sequences of dengue virus type 3 and Japanese encephalitis virus
Ikuo Takashima Hokkaido University	Japanese encephalitis in Hokkaido
Tsutomu Takegami Kanazawa Medical College	Expression of RNA polymerase NS5 of Japanese encephalitis virus in *E. coli*
Mario Tanaka Nagasaki University	Molecular analysis of an attenuated ML-17 and its parental Ja OHO566 strains of Japanese encephalitis virus
Koki Taniguchi Sapporo Medical College	Nucleotide sequence of VP3 gene of a human rotavirus strain K8
Shozo Urasawa Sapporo Medical College	Survey of human rotavirus serotypes in Japan Analysis of neutralizing antigen of human rotavirus using monoclonal antibodies
Koichi Yamanishi Osaka University	Immunity and pathogenesis of hantavirus in mice
Shuzo Yamazaki National Institute of Health Tokyo	Serodiagnosis of small round structured virus (SRSV) infection prevalent in Japan
Kotaro Yasui Tokyo Metropolitan Institute for Neuroscience	Sequence and epitope analysis of Japanese encephalitis virus glycoprotein E

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Joint Scientific Conferences

VIRAL DISEASES PANELS

Date: October 28–30, 1985

Place: Bethesda and Frederick, Maryland

Summary: The scientific sessions on dengue and other arboviral diseases, hemorrhagic fever with renal syndrome and viral gastroenteritis were held at the 19th Joint Panel Meeting on Viral Diseases at the National Institutes of Health in Bethesda during the first 2 days of the Joint Meeting. The final session on rabies was held at the United States Army Medical Research Institute for Infectious Diseases, Fort Detrick, Frederick, Maryland and was combined with a tour of the research laboratories at this facility. Scientific highlights of this meeting included reports of: the cloning of the genomic segments of human rotaviruses using baculovirus vectors and insect cells; the immunogenicity of bovine rotavirus vaccine WC-3 in humans; and evidence that human protection against rotaviruses is serotype specific. Also, reports of the first immunogenicity field trials with rhesus rotavirus vaccine revealed good serologic responses against serotypes 2 and 3 when the dose was 10,000 plaque-forming-units. Continuous cell lines of human monocytes were reported to provide a marker for the virulence of dengue viruses for humans. The complete nucleotide sequence of the reading frame for the 17D strain of yellow fever virus was determined and revealed that it contains all of the known yellow fever proteins. Amino acid sequences of all yellow fever structural proteins were deduced from these nucleotide sequences. Cloning of the full length of the dengue virus type 4 cDNA was reported and RNA sequences were determined to reveal several similarities to other flaviviruses, especially for the nonstructural protein NS-1 which has been reported to be an important immunogen for protection against lethal flavivirus infections. Clones of cDNA for dengue type 2 are being used for molecular epidemiology. Cloning and sequencing of the genome of Japanese encephalitis virus was reported and revealed that, like other arboviruses, the genome is not polycistronic. The construction of a vaccinia virus modified genetically to contain the gene for the envelope glycoprotein of rabies virus was described. This modified vaccinia virus construct successfully immunized raccoons, foxes, and skunks against challenge with street rabies virus.

Date: November 18–20, 1986

Place: Nagasaki, Japan

Summary: The 20th Joint Meeting of the Viral Diseases Panels was held at the School of Tropical Diseases, Nagasaki University in the beautiful and historic city of Nagasaki. Scientific reports included several stimulating papers on the ecology and epidemiology of Japanese encephalitis virus. Information on the field trials for a JE vaccine (partially purified killed virus) in Southeast Asia was also presented. Progress on live, attenuated, dengue virus vaccines was reported. Several papers on nucleotide sequencing of genomes of arboviruses were presented. The coding strategy and nucleotide sequence of segments of the Hantaan virus (HFRS) were reported. New information on seroepidemiology of rotaviruses using more defined antigenic reagents was presented. The use of monoclonal antibodies has enhanced rotavirus serology and identification. Several papers on rabies virus research were presented including an update on oral vaccination studies to control rabies in wildlife and the mapping of functional sites on rabies proteins. An important finding of clinical trials of rotavirus vaccines indicated that the live virus rhesus rotavirus vaccine when administered to infants in a nursery does not spread to unvaccinated children. Reassortant rotavirus vaccines for human serotypes type 1 and 2 have been constructed and are studied as potential candidate vaccines for humans. Studies on the development of subunit rotavirus vaccines using rDNA technology have been initiated.

Date: December 2–4, 1987

Place: Monterey, California

Summary: The 21st Meeting of the Viral Diseases Panels was convened in a combined meeting and symposium with the Immunology Boards at this renowned resort city. The first day of the meeting was a joint symposium with the immunologists with several distinguished presentations including lectures on catalytic antibodies, some current approaches to viral vaccines, transactivating proteins in host cells, immune recognition of rabies virus proteins by T cell clones and the effects of adoptive transfer by these T cells, the antigen binding site and T cell recognition region of Class I histocompatibility antigens, genetic control of nonresponsiveness to hepatitis B surface antigens, viral gastroenteritis, and immunity and pathogenesis of Hantavirus in mice. Ample opportunities for scientist to scientist exchanges and a tour of the Hopkins Marine Station Laboratories were provided. During the remaining 2 days, the Viral Diseases Panels and the Immunology Boards conducted separate scientific sessions in adjoining meeting rooms so that participants could attend easily one session or the other. At the Viral Diseases session an outstanding example of the power of molecular virology was demonstrated by the report that Western equine arbovirus was a natural recombinant of two other arboviruses, Sindbis and Eastern equine encephalitis viruses. This finding was deduced from nucleotide sequences of the three viruses. Several other papers on the molecular biology, pathogenesis and epidemiology of arboviruses were presented including the complete nucleotide sequences of dengue type 3 virus and the enhancement of dengue infection of human monocytes by gamma interferon. New epidemiologic information on hemorrhagic fever with renal syndrome were reported and included preliminary results of ribavirin trials in China. Ribavirin therapy has a beneficial effect when administered within 24 hours of first clinical symptoms. Several presentations on rabies included immune responses to a chick embryo rabies vaccine and the human diploid rabies vaccine and the role of internal antigens in the stimulation of protective responses to rabies antigens. Reports on viral gastroenteritis revealed the remarkable utility of the ELISA with monoclonal antibodies for serotyping human rotaviruses in stool specimens. The latter development promises to accelerate vaccine research. Dr. Frederick Murphy chaired the U.S. Viral Diseases Panel replacing Dr. Thomas Yuill who rotated off.

Date: July 18–20, 1988

Place: Tokyo, Japan

Summary: The 22d Joint Meeting of the Viral Diseases Panels took place at the excellent facilities of the Foundation for the Promotion for Cancer Research. The fourth review of the panel program was initiated at this meeting and, therefore, was scheduled to immediately precede the meeting of the Joint Committee in Tokyo. An excellent scientific program was presented on the epidemiology, molecular biology, immunology and pathogenesis of Japanese encephalitis virus and dengue viruses. A special lecture on HTLV-I-associated myelopathy was presented by Dr. M. Osame. New information was reported on the epidemiology, possible transmission by mites, and immunology of hantavirus infections. And stimulating new results of sero- and molecular epidemiology of rotavirus infections were reported, again emphasizing the advancements made possible by the new biotechnologies. An update on the progress of the rhesus rotavirus vaccines and a report of calicivirus associated diarrheal disease were also presented. Other papers on the biology, molecular structure and genetic mapping of rotaviruses were reported.

Date: August 21–23, 1989

Place: Annapolis, Maryland

Summary: The 23d meeting of the Joint Viral Diseases Panels was convened in the legislative buildings of this state capital and resort city. The Japanese Panel was chaired by Dr. Tasuke Konno, replacing Dr. Akira Oya who was promoted to the Japanese Delegation and Joint Committee. Drs. Joel Dalrymple and Shudo Yamazaki were introduced as new members of the US and Japanese Panels, respectively. The scientific program emphasized arboviral diseases, viral gastroenteritis, hemorrhagic fever with renal syndrome (HFRS), and rabies. The arbovirus session was highlighted by the report of the construction of infectious RNA transcripts from cDNA clones of yellow fever virus 17D strain. Also, significant new information on the protective epitopes of Japanese encephalitis virus was presented. The results of clinical trials for a new live chikungunya virus vaccine were described. Reports of recent epidemics of yellow fever and Lassa fever in Africa were presented. Presentation of viral gastroenteritis included epidemiology studies of rotavirus infections in Bangladesh and the United States, a computerized three dimensional structure of rotaviruses, new strains of rotaviruses, vaccine development and serodiagnosis of small round structured virus similar to the Norwalklike agents of human diarrheas. Rabies virus reports included presentations of cDNA cloning sequences of viral protein genes, viral replication and assembly, cellular immune responses, and potential adenovirus vectors for vaccine development.

U.S.-Japan Cooperative Medical Science Program

History of Program Activities

Program Activity	Initiated	Completed
Meeting of Prime Minister Sato and President Johnson	January 12–13, 1965 Washington, D.C.	—
Joint Planning Committee Meeting	April 19–21, 1965 Tokyo	—
First Join Committee Meeting and Panels' Conference	October 4–7, 1965 Honolulu	—
Subcommittee on Program Review and Planning	1970 Tokyo	
Environmental Symposium	Tokyo	1972
Environmental Panel	1972	
Second Five-Year Report	1974	1975
Binational Symposium	Tokyo	1976
Hepatitis Symposium	Tokyo	1978
Hepatitis Panel	1979	
Third Five-Year Report 1975–1980	1979	1980
Immunology Symposium	Tokyo	1980
Immunology Board	1981	
Parasitic Diseases Symposium	Bethesda	1983
Fourth Five-Year Report 1980–1985	1984	1985
Malnutrition Symposium	Bethesda	1985
AIDS Symposium	Washington, D.C.	1987
AIDS Panel	1987	
Fifth Five-Year Report 1985–1990	1989	1990

History of Panel Reviews
1971–1990

Panel/Board Reviews*	Initiated	Completed
Leprosy and Parasitic	1971	1972
Cholera and Tuberculosis	1972	1973
Malnutrition and Viral	1973	1974
Leprosy and Tuberculosis	1976	1977
Viral and Parasitic	1977	1978
Environmental	1978	1979
Cholera and Malnutrition	1979	1980
Viral and Tuberculosis	1982	1983
Hepatitis and Environmental	1984	1985
Malnutrition	1985	1986
Cholera and Immunology	1986	1987
Leprosy and Tuberculosis	1987	1988
Parasitic and Viral	1988	1989
Hepatitis and Environmental	1990	

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*Formal reviews initiated with the formation of the Subcommittee on Program Review and Planning in 1970.

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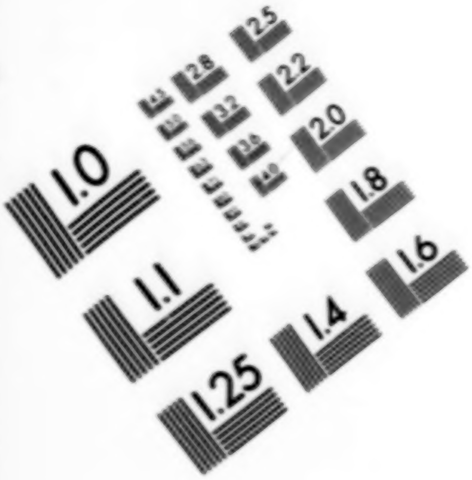

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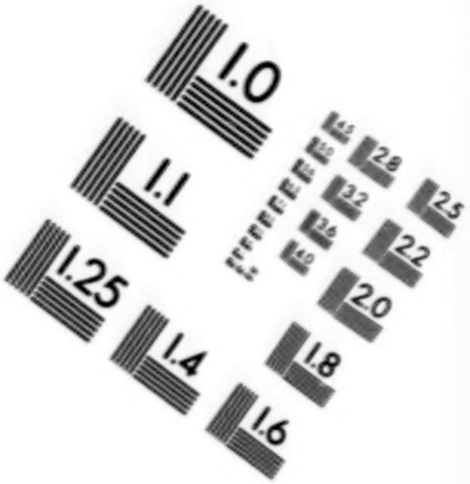

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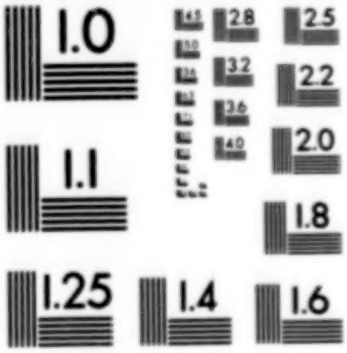

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